A bilayer swellable drug-eluting stent for the treatment of fibrosis-induced ureteral stricture recurrence

Lim, Wei Shan

2018

Lim, W. S. (2018). A bilayer swellable drug-eluting stent for the treatment of fibrosis-induced ureteral stricture recurrence. Doctoral thesis, Nanyang Technological University, Singapore.

<http://hdl.handle.net/10356/73390>

<https://doi.org/10.32657/10356/73390>

NANYANG
TECHNOLOGICAL
UNIVERSITY

**A BILAYER SWELLABLE DRUG-ELUTING STENT FOR THE
TREATMENT OF FIBROSIS-INDUCED URETERAL
STRICTURE RECURRENCE**

LIM WEI SHAN

SCHOOL OF MATERIALS SCIENCE AND ENGINEERING

2017

**A BILAYER SWELLABLE DRUG-ELUTING STENT FOR THE
TREATMENT OF FIBROSIS-INDUCED URETERAL
STRICTURE RECURRENCE**

LIM WEI SHAN

SCHOOL OF MATERIALS SCIENCE AND ENGINEERING

A thesis submitted to the Nanyang Technological University
in partial fulfillment of the requirement for the degree of
Doctor of Philosophy

2017

Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research and has not been submitted for a higher degree to any other University or Institution.

03 August 2017
Date

.....
Lim Wei Shan

Abstract

Ureteric stricture is a long-term condition that can lead to the obstruction in the urinary tract, leading to hydronephrosis and kidney failure. Current therapies are invasive and ineffective in managing stricture recurrence following stenting. It is hypothesized that a bilayer swellable drug-eluting stent (BSDES) that can co-opt with the urothelium for the localized and sustained delivery of drugs into the diseased section can reduce or eliminate stricture recurrence.

The BSDES comprises of two layers of coatings. The first layer consists of mitomycin C (MMC) and 70/30 poly-L-lactide-co-caprolactone (PLC). The second layer consists of polyethylene glycol diacrylate (PEGDA) hydrogel. The first layer is needed for sustained release of drug while the second layer is required for close contact with urothelial wall.

This thesis examines the development, fabrication, characterization and evaluation of a BSDES, the results achieved as below:

Firstly, mass loss and rheological measurements identified 10% w/v PEGDA hydrogel as the candidate hydrogel concentration since there was no significant degradation and it could retain the largest mechanical strength over the 4 weeks. Secondly, plasma treatment (100W, 5 min) was found to be effective in improving the interfacial adhesion between the hydrogel and polymer, allowing the hydrogel to adhere on the stent over the 4 weeks.

Moreover, drug release studies were conducted in the static conditions over 1 month. Tunable drug release was demonstrated in the static condition by varying the polymer thickness and drug concentration. In addition, the drug released in the static condition was well above the minimum effective concentration to inhibit HBdSF (0.01 ug/mL). An in-vitro transwell cell study showed that the drug eluted from the coated stent was able to inhibit human bladder stroma fibroblasts (HBdSF) in-vitro.

To better understand the effect of hydrogel on drug release and transfer of BSDES, the release study of BSDES was conducted in both in-vitro dynamic fluid flow and porcine ureteric tissue ex-vivo dynamic fluid flow condition. The PEGDA hydrogel gave rise to greater drug release in the dynamic fluid flow condition over the 1-week period but this difference was not observed in the static condition. Moreover, PEGDA hydrogel was found to augment the amount of drug transport into the explanted porcine ureteric tissues ex-vivo. These observations were likely due to the high equilibrium water content hence promoting the drug release.

Furthermore, the pilot in-vivo study successfully demonstrated the feasibility of the device in the porcine model. The device was successfully inserted by using the same current clinical stenting methods. The hydrogel swelled and co-opted with the urothelium which MMC was locally delivered to the targeted ureteric section. No hydronephrosis or systemic toxicity was detected. Hydrogel remained intact on the stent.

In conclusion, a BSDES prototype was successfully fabricated and tested in this project, and has the potential to treat and manage ureteric stricture recurrence.

Acknowledgements

The author would like to express his deepest gratitude to **Professor Subbu Venkatraman** for the opportunity to undertake this project and his patient supervision.

He would also like to express his heartfelt thanks to his mentors **Dr Huang Yingying** and **Dr William Birch** for their guidance in the various aspects of the project.

Besides, he would like to thank the clinician collaborators **Dr Chong Tsung Wen** and **Dr Kenneth Chen** for their clinical advice. They have also helped in the implantation of the coated and uncoated ureteral stents into the porcine as well as the harvesting of the ureters for tissue analysis. To add on, the author would also like to express thanks to the technicians at SingHealth Experimental Centre (SEMC) for assisting with the handling of the pigs, including injecting anesthetics and withdrawing blood and serum.

Moreover, the author would like to express his appreciation towards **Dr Lee Bae Hoon** for the synthesis and NMR characterization of the PEGDA hydrogel and his expert advice on the material.

Apart from that, the author would like to give thanks to **Dr Anthony Ng Herr Cheung** for the expert advice on mitomycin C.

To add on, he would like to thank **Dr Gordon Xiong Minru** for the guidance in works such as cell culture and dynamic fluid flow experiment.

Also, he would like to thank **Mr Abhijit Salvekar Vijay**, a PhD colleague in the same lab for the collaboration with the designing prototypes for characterization.

Besides, the author would like to give thanks to several of his fellow students (Final Year Project, Internship etc.) who have helped to contribute to the progress of the project,

especially for Er Pei Shan who has assisted in the experiments involving shear adhesion delamination test and Chin Jia Wen for assisting much with the rheological measurements of the PEGDA hydrogels.

Furthermore, the author would like to thank Agency for Science, Technology and Research (A*STAR) for the graduate scholarship, and the support of his family for the encouragement during the challenging days of his PhD.

Last but not least, the author would like to express gratitude towards all laboratory staffs, technicians and colleagues in School of Materials Science and Engineering who have lent a helping hand throughout the 4 years which have made learning fruitful and fun.

Table of Contents

Abstract	i
Acknowledgements	iii
Table of Contents	v
Table Captions	xii
Figure Captions	xiii
Abbreviations	xx
Chapter 1 Introduction	1
1.1 Problem Statement	2
1.2 Proposed Solution	3
1.3 Hypotheses and Objectives	4
1.4 Scope of Work	5
1.5 Dissertation Overview	7
1.6 Findings and Outcomes.....	8
References.....	9
Chapter 2 Literature Review	11
2.1 Ureteral Stricture	12
2.1.1 Physiology of the Ureter	12
2.1.2 Etiology and Disease State of Ureteral Stricture Condition	13
2.1.3 Current Treatment Methods and Challenges	14

2.1.4	Ureteral Stent	15
2.2	Emerging Research and Innovation	16
2.2.1	Enhancement of Drug Transport Across the Urothelium	16
2.2.2	Drug-eluting Stents	18
2.3	Drug Delivery Systems	19
2.3.1	Types of Drug Delivery Systems	19
2.3.2	Drug Release Kinetics and Models	21
2.3.3	Sustained and Controlled Drug Delivery	23
2.4	Materials Selection.....	24
2.4.1	Ureteral Stent: Polyurethane (PU)	24
2.4.2	Drug: Mitomycin C (MMC)	25
2.4.3	Drug-carrier Polymer: Poly-L-Lactide-co-Caprolactone (PLC).....	26
2.4.4	Hydrogel: Polyethylene Glycol Diacrylate (PEGDA)	27
	References	28
Chapter 3	Experimental Methodology	35
3.1	Materials	36
3.2	Preparation and Fabrication of Bilayer Swellable Drug-eluting Ureteral Stent	36
3.2.1	Spray-coating	37
3.2.2	Dip-coating	38
3.2.3	Synthesis of Polyethylene Glycol Diacrylate (PEGDA)	39
3.2.4	Synthesis of Polyethylene Glycol Diacrylate Hydrogel (PEGDA)	40
3.2.5	Spin-coating	42
3.2.6	Plasma Treatment.....	42

3.2.7	Customized setup for the Coating of Polyethylene Glycol Diacrylate (PEGDA) Hydrogel onto Stent via Photo-crosslinking	43
3.3	Material Characterization	45
3.3.1	Thermogravimetric Analysis (TGA)	45
3.3.2	Scanning Electron Microscopy (SEM)	46
3.3.3	Nuclear Magnetic Resonance (NMR)	47
3.3.4	Measurements of Gel Fraction	48
3.3.5	Measurements of Equilibrium Water Content	48
3.3.6	Measurements of Swelling Ratio	48
3.3.7	Measurements of Mesh Size	49
3.3.8	Mass Loss Measurements of Hydrogel	50
3.3.9	Rheological Measurements of Hydrogel	51
3.3.10	Static Water Contact Angle Measurements	54
3.3.11	Measurements of Adhesive Strength between Stent and PEGDA Hydrogel via Pull-off Test	54
3.3.12	Preliminary Tactile Assessment of Long-term Adhesion	56
3.3.13	Atomic Force Microscopy (AFM)	57
3.4	Cellular Studies	58
3.4.1	Cell Proliferation Assays	58
3.4.2	Transwell Migration Assays	60
3.5	Characterization of Drug Release	62
3.5.1	High-Performance Liquid Chromatography (HPLC)	62
3.5.2	Loading Efficiency of MMC onto Stent	64
3.5.3	Stability of MMC	65
3.6	Design of Customized Setups for the Simulation of Dynamic Fluid Flow Condition	65

3.6.1	In-vitro Drug Release	66
3.6.2	Ex-vivo Drug Transport	68
3.6.3	Solid Phase Extraction (SPE).....	70
3.6.4	Liquid Chromatography Mass Spectroscopy (LCMS)	72
3.7	In-vivo Pilot Study	73
3.7.1	Fabrication of the Working Prototype	74
3.7.2	Pre-implantation Procedures	75
3.7.3	Implantation Procedures	75
3.7.4	Post-study Procedures	75
3.7.5	Feasibility Analysis.....	76
3.8	Statistical Analysis.....	77
	References.....	77

Chapter 4 Fabrication and Characterization of Bilayer Swellable Drug-eluting Ureteral Stent

4.1	Introduction.....	84
4.2	Experimental Methods	84
4.3	Fabrication and Characterization MMC Loaded Layer	85
4.3.1	Thermogravimetric Analysis (TGA) of MMC-coated Stent (Prepared by Dip-coating)	85
4.3.2	Surface Morphology of MMC-coated Stent.....	86
4.3.3	Loading Efficiency of MMC onto Stent	87
4.4	Synthesis and Characterization of Polyethylene Glycol Diacrylate (PEGDA) Hydrogel Layer	88
4.4.1	Nuclear Magnetic Resonance (NMR) of PEGDA	89
4.4.2	Thermogravimetric Analysis (TGA) of PEGDA Hydrogel.....	90

4.4.3	Gel Fraction of PEGDA Hydrogel.....	91
4.4.4	Equilibrium Water Content of PEGDA Hydrogel	91
4.4.5	Swelling Ratio of PEGDA Hydrogel.....	92
4.4.6	Mesh Size of PEGDA Hydrogel	93
4.4.7	Mass Loss of PEGDA Hydrogel.....	94
4.4.8	Rheological Properties of PEGDA Hydrogel	95
4.4.9	Coating of PEGDA Hydrogel onto Stent.....	96
4.5	Surface Plasma Treatment of Stent to Improve Adhesion of PEGDA Hydrogel on PLC-coated Stent	97
4.5.1	Static Water Contact Angles of Spin-coated Polymer Films	98
4.5.2	Force Measurements of Adhesion Strength of PEGDA Hydrogel on the Stent via Pull-off Test	99
4.5.3	Preliminary Tactile Assessment of Long-term Adhesion	99
4.5.4	Effect of Plasma on Surface Roughness	101
4.6	Discussions and Conclusions.....	102
	References.....	104
 Chapter 5 Characterization of the Drug Release Rates in Static Condition for the Inhibition of Human Stromal Bladder Fibroblasts (HBdSF) Proliferation		
5.1	Introduction.....	108
5.2	Experimental Methods	108
5.3	Assessment of the Drug-eluting Stent in Inhibiting HBdSF Proliferation	108
5.3.1	Cell Proliferation Assay to Find the Minimum Effective Concentration and Growth Inhibitory Concentration of MMC to Inhibit HBdSF Proliferations	109

5.3.2	Transwell Migration Assay to Evaluate the Feasibility of Drug-eluting Stent to Inhibit HBdSF Proliferations.....	110
5.4	Tuning and Characterizing Drug Release	111
5.4.1	Evaluation of the Stability of MMC	111
5.4.2	Effect of Polymer Thickness on Drug Release	112
5.4.3	Effect of Drug Loading on Drug Release	114
5.4.4	Evaluation of Daily Drug Dosage.....	117
5.4.5	Modelling and Understanding the Kinetics and Mechanism of Drug Release	118
5.4.6	Effect of PEGDA Hydrogel on Drug Release	121
5.5	Discussions and Conclusions.....	122
	References.....	123
 Chapter 6 Characterization of the Drug Release in In-vitro Dynamic Fluid Flow Condition and Evaluation of the Coated Stent to Enhance Transport of Drug into Explanted Porcine Urothelial Tissues Ex-vivo.....		
6.1	Introduction.....	128
6.2	Experimental Methods	128
6.3	Elucidation of the Drug Release in In-Vitro Dynamic Fluid Flow Condition.....	128
6.3.1	Effect of PEGDA Hydrogel on Drug Release.....	128
6.4	Elucidation of the Coated Stent to Enhance Transport of Drug into Explanted Porcine Urothelial Tissues Ex-vivo.....	131
6.4.1	Effect of PEGDA Hydrogel on Drug Transport into Explanted Porcine Ureteral Tissues	131
6.5	Discussions and Conclusions.....	133
	References.....	136

Chapter 7	In-vivo Pilot Study	139
7.1	Introduction	140
7.2	Experimental Methods	140
7.3	Evaluation of Hydrogel Co-aptation with Urothelial Tissues	141
7.4	Evaluation of Drug Uptake in Tissues	143
7.5	Evaluation of Systemic Toxicity	145
7.6	Discussions and Conclusions	146
	References	147
Chapter 8	Overall Conclusions and Future Work	149
8.1	Overview of Research Findings	150
8.2	Implications of Research Findings	152
8.3	Future Studies and Future Research Direction	154
	References	156
	Appendix	157
	Patent, Publication and Conferences	185

Table Captions

Table 3.1	Different test conditions for transwell migration assay	61
Table 5.1	Comparison of C_o and C_s for different drug dosages	116
Table 5.2	Computation of the degree of burst	119
Table 6.1	Cumulative drug release (%) for samples coated with and without hydrogel under static and dynamic fluid flow conditions	135
Table 7.1	Drug delivered to selected segments.....	144
Table 7.2	Comparison between drug transported (%) in <i>ex-vivo</i> and <i>in-vivo</i> models	144

Figure Captions

Figure 1.1	Ureteral stricture	2
Figure 1.2	Schematic diagram showing bilayer coated stent design.....	3
Figure 2.1	Urinary tract system.....	12
Figure 2.2	Different layers of a ureteric wall	13
Figure 2.3	Commercially available ureteral stent	15
Figure 2.4	Schematic diagrams showing matrix and reservoir systems.....	20
Figure 2.5	Chemical structures of monomer L-lactide (top left), monomer caprolactone (top right) and PLC (bottom).....	27
Figure 2.6	Chemical structure of PEGDA.....	27
Figure 3.1	Schematic diagram of spray-coating.....	37
Figure 3.2	Schematic diagram of dip-coating	38
Figure 3.3	Synthesis of PEGDA from PEGOH and acryloyl chloride	39
Figure 3.4	Formation of radicals upon UV irradiation of Irgacure-2959.....	40
Figure 3.5	Schematic illustration of the formation of PEGDA hydrogel.....	40
Figure 3.6	Dry PEGDA hydrogel (left) and wet PEGDA hydrogel (right)	41

Figure 3.7	Schematic diagram illustrating the method to coat PEGDA hydrogel onto the stent	44
Figure 3.8	Schematic diagram showing the intended working prototype with the hydrogel coating designed with tapered ends	45
Figure 3.9	A crosslinked hydrogel depicting its mesh size	49
Figure 3.10	Schematic diagram of a rheometer	52
Figure 3.11	Schematic diagram of the shear adhesion failure analysis experiment.....	55
Figure 3.12	Coated stent sample to be assess for long-term adhesion	56
Figure 3.13	Preliminary tactile assessment, showing that the right fingers moving along the stent/hydrogel while the left fingers clamping the stent	56
Figure 3.14	Principles of AFM.....	57
Figure 3.15	Conversion of Resazurin to Resorufin via NADH	59
Figure 3.16	Schematic illustration of <i>in-vitro</i> dynamic fluid flow model.....	67
Figure 3.17	Actual illustration of <i>in-vitro</i> dynamic fluid flow model.....	67
Figure 3.18	Schematic illustration of <i>ex-vivo</i> dynamic fluid flow model	69
Figure 3.19	Actual illustration of <i>ex-vivo</i> dynamic fluid flow model	70
Figure 3.20	Schematic diagram showing the coated stent prototype	74

Figure 4.1	Spray-coated stents	85
Figure 4.2	TGA result of drug-coated stent	86
Figure 4.3	Surface morphology and cross-section of coated stent samples	87
Figure 4.4	Loading efficiency of drug in PLC coated in stent	88
Figure 4.5	NMR peaks showing formation of PEGDA	89
Figure 4.6	NMR diagram showing acrylation of 80.6%	89
Figure 4.7	TGA result of PEGDA hydrogels	90
Figure 4.8	Gel fraction of PEGDA hydrogels	91
Figure 4.9	Equilibrium water content of PEGDA hydrogels	92
Figure 4.10	Swelling ratio of PEGDA hydrogels.....	93
Figure 4.11	Mesh size of PEGDA hydrogels	94
Figure 4.12	Mass loss measurements of PEGDA hydrogels.....	95
Figure 4.13	Rheological measurements of PEGDA hydrogels	96
Figure 4.14	A section of the bilayer coated stent sample.....	97
Figure 4.15	Cross-section of the coated stent, depicting the drug and hydrogel coatings	97

Figure 4.16	Effect of plasma time on contact angles	98
Figure 4.17	Effect of plasma time on forces of adhesion.....	99
Figure 4.18	Effect of plasma time on long term adhesion of hydrogel on stent samples	101
Figure 4.19	Effect of plasma time on surface roughness	102
Figure 5.1	Effect of MMC concentrations on relative HBdSF cell count.....	109
Figure 5.2	Amount of inhibition (%) vs Log [MMC]	110
Figure 5.3	Effect of drug-eluting stent samples on the inhibition of HBdSF in transwell migration study.....	111
Figure 5.4	Stability of MMC.....	112
Figure 5.5	Effect of polymer thickness on drug released (%).....	114
Figure 5.6	Effect of polymer thickness on drug released (ug)	114
Figure 5.7	Effect of drug loading on drug released (%).....	115
Figure 5.8	Effect of drug loading on drug released (ug)	116
Figure 5.9	Amount of drug released daily for different polymer thickness	117
Figure 5.10	Amount of drug released daily for different drug loading.....	118
Figure 5.11	Cumulative drug released (%) for different polymer thickness vs square	

	root of time in static condition	120
Figure 5.12	Cumulative drug released (%) for different drug loading vs square root of time in static condition.....	120
Figure 5.13	Smooth surface morphology of MMC/PLC coatings post-release studies	121
Figure 5.14	Effect of PEGDA hydrogel on drug released (%) in static condition.....	122
Figure 6.1	Effect of PEGDA hydrogel on drug released (%) in dynamic fluid flow condition	130
Figure 6.2	Effect of PEGDA hydrogel on drug released (ng) in dynamic fluid flow condition	130
Figure 6.3	Cumulative drug released (%) vs square root of time in dynamic fluid flow condition	131
Figure 6.4	Effect of PEGDA hydrogel on the drug amount delivered to porcine ureteric tissues <i>ex-vivo</i> in dynamic fluid flow condition	133
Figure 6.5	Cross-section view of the bilayer coated section	135
Figure 7.1	Bilayer coated stent before implantation	140
Figure 7.2	Bilayer coated stent before implantation (close-up view)	140
Figure 7.3	Harvested stent in ureter after 1 day implantation	141
Figure 7.4	Harvested stent in ureter after 1 day implantation (close-up view)	141

Figure 7.5	X-ray image showing the hydrogel has swelled in the ureter, hence causing the accumulation of contrast media at the region just before the gel segment (top); X-ray image showing that the contrast can flow through the stent hollow lumen to reach the bladder, as an important route of passage from kidney to bladder without potentially causing urine backflow due to obstruction in ureter (hydronephrosis) (bottom)142
Figure 7.6	Graph depicting the drug delivered to selected segments.....144
Figure 7.7	Drug (MMC) level in porcine blood before and after study146

Abbreviations

AFM	Atomic Force Microscopy
DMSO	Dimethyl Sulfoxide
DPBS	Dulbecco's Phosphate Buffered Saline
EDTA	Ethylene Diamine Tetra Acetic Acid
ETO	Ethylene Oxide
HBdSF	Human Bladder Stroma Fibroblast
HPLC	High-Performance Liquid Chromatography
IS	Internal Standard
LCMS	Liquid Chromatography Mass Spectrometry
MEC	Minimum Effective Concentration
MMC	Mitomycin C
NMR	Nuclear Magnetic Resonance
PCL	Polycaprolactone
PEGDA	Polyethylene Glycol Diacrylate
PEGOH	Polyethylene Glycol Diol
PLA	Polylactic Acid
PLC	Poly-L-lactide-co-Caprolactone
PFM	Porfiromycin
PU	Polyurethane
SEM	Scanning Electron Microscopy
SPE	Solid Phase Extraction
TGA	Thermogravimetric Analysis

Chapter 1

Introduction

This chapter describes the problem which the thesis addresses. The disease state to overcome is ureteric stricture recurrence. Current methods to overcome the problem are ineffective and highly invasive. A new solution with the use of bilayer swellable drug-eluting stent (BSDES) is proposed. Hypotheses and objectives are laid out clearly. The scope of work is clearly stated. Moreover, the dissertation overview is depicted in which the gist of each chapter is described. Lastly, the novel findings and outcomes generated from the research are described. Briefly, it is found that the successfully fabricated BSDES prototype is able to inhibit human bladder stroma fibroblasts in-vitro and dynamic fluid flow studies show that the hydrogel augments the drug transport in porcine ureteric tissues ex-vivo. Moreover, in-vivo study show that the device is feasible in porcine model without signs of side effects.

1.1 Problem Statement

Ureteral stricture is a chronic urological disease which results in obstruction to urine flow from kidney to bladder (Fig 1.1). This can result in hydronephrosis and potential loss of a functioning kidney.¹ The disease is mainly resulted from the excessive deposition of collagen due to scarring.² Standard of care comprising endoscopic full-thickness incision of the ureteric wall, and placement of an indwelling stent for up to 4 to 6 weeks, is not always successful nor able to produce durable favorable outcomes, and is associated with recurrent obstruction.^{3,4} Therefore, there is an urgent need for a solution that prevents recurrent stricture, improves the quality of patient care and reduces patient trauma and the high medical costs.

Fig 1.1 Ureteric stricture

There are two key underlying challenges due to the nature of ureteric tract. First, a ureteral stent commonly used has smaller diameter at 1.67 to 2 mm compared to the ureter in an adult which usually ranges in the diameter of 3 to 5 mm. Secondly, the urothelial lining is one of the most drug-impermeable barriers.⁵ Therefore, it is crucial that the proposed solution will swell/expand in response to the urine absorption and achieve close apposition with the urothelial lining so that the drugs can be effectively delivered laterally into the diseased ureteric tissues.

1.2 Proposed Solution

It is proposed that a swellable drug-eluting ureteral stent (Fig 1.2) that can be implanted post-surgical intervention as a prophylactic treatment to prevent ureteric stricture recurrence. Since a ureteric stent is commonly implanted after surgical interventions, by implanting a drug-eluting stent in place of a conventional stent, it would not alter the clinical flow of care. The technology aims to deliver drug to the proximate diseased ureteric section to inhibit fibroblast proliferation due to scarring. The device is a conventional polyurethane ureteric stent coated with 2 layers of coatings: The first layer comprising of drug-encapsulated polymer and the second layer comprising of a swellable hydrogel. Candidate materials selected for this thesis are as follows: Mitomycin C (MMC) as the anti-fibrotic drug, poly-L-lactide-co-caprolactone (PLC) as the polymer that encapsulates the drug and polyethylene glycol diacrylate (PEGDA) as the hydrogel.

Fig 1.2 Schematic diagram showing bilayer coated stent design

1.3 Hypotheses and Objectives

It is hypothesized that:

- 1) A multi-layer swellable coating can be applied to ureteric stents, with no delamination.**
- 2) The swollen hydrogel layer would enhance the localized and sustained drug delivery to the urothelium.**

In elaboration, the hypotheses are formed on the following basis. Firstly, there have already been many coatings that have been successfully applied onto the ureteral stents for various functionalities, such as silver, teflon, heparin, diamond-liked carbon coatings and hydrophilic coatings to prevent biofilm formation. Drugs such as triclosan and ketorolac have also been successfully applied on ureteral stents to reduce bacterial adhesion and pain during stenting period.⁶ None of these technologies are swellable and none of these coatings are applied explicitly to enhance contact between the stent and the tissue of the ureteric wall. Not surprisingly, the applied coatings have not been reported to undergo delamination while in use. Secondly, Liatsikos and co-workers have successfully demonstrated that a paclitaxel-eluting balloon can deliver drugs into the urothelium following 12 hours of close contact between the balloon and urothelium⁷ and there have been studies that demonstrate that the increase of drug residence time on the target urothelium can increase the drug uptake.⁸ However, this option is not yet available in the clinic and because of its non-hollow nature, it is impractical to leave the balloon in the ureteric tract for 12 hours as this might obstruct the urine flow. A ureteric stent, on the other hand, is designed to have a hollow lumen for urine passage and it is common for the stent to be left in ureter for as long as 4 to 6 weeks.

To validate the hypotheses, the following objectives are laid out:

1) Develop and fabricate a bilayer swellable drug eluting stent prototype. A BSDES comprises of two layers of coatings. The first layer is a drug-encapsulated polymeric layer, and the second layer is a hydrogel, layer. Hence it is necessary to first investigate the integrity of hydrogel and the adhesion of hydrogel and polymeric layer. *(This is discussed in Chapter 4).*

2) Study the *in vitro* release behavior and drug transfer from BSDES in static, simulated dynamic, and *ex-vivo* dynamic conditions. PEGDA hydrogel is designed for the purpose of mediating the drug transfer. Therefore, it is crucial to evaluate if drug delivery can be manipulated by the hydrogel under static setting, the dynamic fluid flow condition and in *ex-vivo* setting. *(This is discussed in Chapters 5 and 6).*

3) Investigate the feasibility of BSDES in porcine model. It is critical to test the lateral transfer of drugs to the specific selected section of the ureter *in-vivo* without causing systemic toxicity and hydronephrosis. *(This is discussed in Chapter 7).*

1.4 Scope of Work

1) Develop a method to fabricate the bilayer coated stent prototype.

Briefly, this can be achieved by using spray-coating to load the first layer of drug onto stent, then followed by the photo-crosslinking method to coat the hydrogel over first coating using a plastic tubing.

2) Synthesize PEGDA hydrogel that can swell and maintain mechanical stability over one month.

This can be achieved by evaluating the swelling ratio of the hydrogels. No targeted value of the swelling ratio is set, so long as the hydrogel is able to achieve close apposition with the ureter in *in-vivo* setting. To meet this target, the plastic tubing used during photo-crosslinking for the hydrogel coating has a diameter of at least 4.5 mm since ureter diameter measures at around 3 - 5 mm. The mechanical stability of

the hydrogel can be assessed using rheological measurements to obtain storage (elastic) modulus over the 1 month period.

3) Adhere the PEGDA hydrogel firmly onto the coated stent without delamination for one month.

This is achieved by plasma treating the stent coatings to impart hydrophilicity, so that the hydrophilic hydrogel can adhere onto it with greater interfacial adhesive forces. No chemical methods are used so that the drugs mitomycin C is not chemically influenced.

4) Inhibit human bladder stroma fibroblasts *in-vitro* using the drugs eluted from the device.

Following the plasma treatment and UV irradiation, the bioactivity of the drugs on the coating might be affected. Hence, it is important to evaluate if the drugs are able to inhibit the fibroblasts using a transwell migration experiment in which the coated stent prototypes were placed on a transwell, separate from the cells seeded below. Moreover, it is also important to show that the other materials such as 0.1% photoinitiator, PEGDA and PLC are cyto-compatible and not responsible for the cell death.

5) Augment the drug permeation through the urothelium using PEGDA hydrogel which can swell and contact with ureter in *ex-vivo* setting under dynamic fluid flow condition.

It is expected that the drugs would be washed away before they can reach the target urothelium, hence PEGDA hydrogel is designed for the purpose of mediating the drug transfer. Therefore, it is crucial to evaluate if drug permeation and delivery can be augmented by the close apposition of the hydrogel with the urothelium under the dynamic fluid flow condition in *ex-vivo* setting.

6) Achieve the lateral transfer of drugs to the specific selected section of the ureter *in vivo* without causing systemic toxicity and hydronephrosis.

This is assessed using porcine as the animal model, in which the stents were placed in the ureter and then after a day, the stents removed and ureters harvested for pharmacokinetic analysis.

1.5 Dissertation Overview

The thesis include 8 separate chapters.

Chapter 1 describes the problem statement and provides the motivation for the research. The hypotheses, objectives and scope of work are also outlined. A brief overview of the findings and outcomes of the studies are also highlighted.

Chapter 2 reviews the literature concerning the etiology of the disease state of ureteric stricture and the drawback of the current treatment methods. Also, relevant and interesting emerging research efforts in related fields are described. In addition, the review will also shed light on the effectiveness of using hydrogel and stents to deliver drugs. Emphasis is given to the enhancement of drug transport to tissues using hydrogels. Finally, concepts about drug release kinetic and mechanisms are reviewed.

Chapter 3 discusses the principles of the synthesis and characterization techniques. Also, the rationale for the choice of experimental methods used is described followed by the detailed protocols of how the technique is carried out.

Chapter 4 elaborates on the fabrication of the coated stent prototype using spray-coating and photo-crosslinking technique. The synthesis and characterization of PEGDA hydrogel are also reported. The effect of plasma treatment and the characterization methods to evaluate the adhesion of the hydrogel on the stent was also discussed.

Chapter 5 elaborates on the characterization of the drug release of the coated stent prototype in static condition. This includes a transwell migration cellular assay to

assess the feasibility of the drug-eluting device in the inhibition of human bladder stroma fibroblasts *in-vitro*. The drug release kinetics and mechanisms are also elucidated where the effects of polymer thickness, drug concentration and PEGDA hydrogel are investigated.

Chapter 6 elaborates on the characterization of the drug release of the coated stent prototype in dynamic fluid flow condition. The effect of PEGDA hydrogel on drug release is investigated and compared to the results in static condition. Lastly, an *ex-vivo* model is set up to investigate the effect of PEGDA hydrogel on facilitating the device in the enhancement of drug transfer into explanted porcine ureteric tissues.

Chapter 7 discusses the proof-of-concept studies which investigate the feasibility and safety of the device in a porcine model. The ureteric tissue samples are collected for pharmacokinetic analysis to evaluate if the drugs are transported into the targeted segment. Moreover, blood samples, periureteric fat tissues, creatinine levels and the condition of the kidneys are also analyzed for signs of systemic toxicity and hydronephrosis, characterized by swelling of the kidney calyx.

Chapter 8 summarizes the findings in the conclusions and provides an insight about the implications and novelty of the research. It reiterates the extent to which the hypotheses are proven and evaluates if the design requirements have been met. Finally, opportunities and strategies for future work are presented.

1.6 Findings and Outcomes

This research led to numerous novel learning outcomes:

1. The bilayer coated stent has been successfully fabricated in the lab. The desired target design features were met, with the drug-encapsulated polymer and hydrogel coated over the stent surface, and the diameter of the coated section swelling to a range of 5 - 5.5 mm in diameter, close to the diameter of the ureter in adult. Plasma treatment (100W, 5 min) was found to be effective in adhering the hydrogel on the stent in the 4-weeks study.

2. Drugs eluted from stent were found to be active and effective in inhibiting human bladder stroma fibroblasts *in-vitro*. The PEGDA hydrogel and PLC materials were found to be cyto-compatible with the cells and photo-initiator at 0.1% w/v was not toxic to the cells *in-vitro*. The release rate of drug eluted from stent can be finely tuned by varying polymer thickness and drug concentration. Hydrogel did not contribute to significant difference in drug release rate over the 4 weeks study in the static condition, except within the first hour. This is likely because the drugs have equilibrated between hydrogel and coated polymer reservoir.

3. A customized dynamic fluid flow model was successfully designed and created to simulate the drug release and transport. Hydrogel was found to have increased the drug released from device and the amount delivered to porcine ureteric tissues *ex-vivo* under the dynamic fluid flow conditions.

4. The coated stent tested *in-vivo* demonstrates success in transferring the drugs to the targeted ureteric section over 1-day preliminary study in porcine model. No stent migration, hydronephrosis and systemic toxicity was recorded.

References

1. Herati, A. S.; Atalla, M. A., Distal Ureteral Strictures. In *Smith's Textbook of Endourology*, Wiley-Blackwell: 2012; pp 472-480.
2. Ayyildiz, A.; Nuhoglu, B.; Gulerkaya, B.; Caydere, M.; Ustun, H.; Germiyanoglu, C.; Erol, D., Effect of intraurethral Mitomycin-C on healing and fibrosis in rats with experimentally induced urethral stricture. *International journal of urology : official journal of the Japanese Urological Association* **2004**, *11* (12), 1122-6.
3. Fasihuddin, Q.; Abel, F.; Hasan, A. T.; Shimali, M., Effectiveness of endoscopic and open surgical management in benign ureteral strictures. *JPMA. The Journal of the Pakistan Medical Association* **2001**, *51* (10), 351-3.
4. Benjamin Newell Breyer, M., MS; Chief Editor: Bradley Fields Schwartz, DO, FACS (2017). "Ureteral Stricture Treatment & Management." Retrieved 28 February 2017, from <http://emedicine.medscape.com/article/442469-treatment>.

5. Douglass, L.; Schoenberg, M., The Future of Intravesical Drug Delivery for Non-Muscle Invasive Bladder Cancer. *Bladder Cancer* **2016**, 2 (3), 285-292.
6. Yang, L.; Whiteside, S.; Cadieux, P. A.; Denstedt, J. D., Ureteral stent technology: Drug-eluting stents and stent coatings. *Asian Journal of Urology* **2015**, 2 (4), 194-201.
7. Liourdi, D.; Kallidonis, P.; Kyriazis, I.; Tsamandas, A.; Karnabatidis, D.; Kitrou, P.; Spyroulias, G. A.; Kostopoulou, O. N.; Marousis, K.; Kalpaxis, D. L.; Goumenos, D. S.; Liatsikos, E., Evaluation of the distribution of Paclitaxel by immunohistochemistry and nuclear magnetic resonance spectroscopy after the application of a drug-eluting balloon in the porcine ureter. *Journal of endourology* **2015**, 29 (5), 580-9.
8. Tyagi, P.; Wu, P.-C.; Chancellor, M.; Yoshimura, N.; Huang, L., Recent Advances in Intravesical Drug/Gene Delivery. *Molecular pharmaceuticals* **2006**, 3 (4), 369-379.

Chapter 2

Literature Review

This chapter addresses the research gaps which mainly focuses on the highly drug-impermeable urothelium and reviews the literature concerning the etiology of the disease state of ureteric stricture and the drawback of the current treatment method being ineffective and highly invasive. Also, relevant and interesting emerging research efforts in related fields are described. Furthermore, the review also sheds light on the perspective of using drug delivery stents. Emphasis will be given to the enhancement of drug transport to tissues using hydrogels. Finally, concepts about drug release kinetics and mechanisms are reviewed.

2.1 Ureteral Stricture

The key physiological components of a ureter in the urinary system are pointed out, and the etiology and disease state of the ureteric stricture are reviewed. The current treatment methods are presented with their limitations. Finally, the ureteral stent device, a commonly implanted device after surgical interventions, is explained and several emerging research efforts related to the field are also presented.

2.1.1 Physiology of the Ureter

The ureter is a tube that runs from the kidney to the bladder, measuring at around 8 - 10 inches long and 3 - 4 mm wide (Fig 2.1).¹ It functions to transport excretory waste products and fluids from the kidney to the bladder in order to maintain homeostasis.² In the event that the fluid flow in the ureter is blocked, the patients need to undergo medical treatments for the removal of the obstruction.

Fig 2.1 Urinary tract system

The ureteric wall is made up of 3 layers of tissues, namely the fibrous coat, muscular coat and mucosa. The fibrous coat lies in the outermost layer and serves as the support for the ureter. The muscular coat consists of smooth muscles which direct the peristaltic movement of the fluid in the ureter. The mucosa in the innermost layer of

the ureteric lining is made of transitional epithelium, which helps to protect and lubricate the ureter (Fig 2.2).³

Fig 2.2 Different layers of a ureteric wall

This layer of epithelium is stratified, having made up of a layer of "umbrella cells" at the luminal surface. The umbrella cells are covered by rigid plaques, comprising of uroplakins which gives rise to the impermeability of the ureteric lining. Furthermore, the impermeability is also increased by the presence of glycosaminoglycans (GAGs) on the urothelium that form a tight barrier. The impermeability of the urothelium functions to prevent the leakage of toxic metabolites into the circulatory system for prolonged period of time, however, it poses a significant challenge to the delivery of therapeutic drugs for effective treatments.⁴

2.1.2 Etiology and Disease State of Ureteral Stricture Condition

Ureter stricture is the undesirable and abnormal narrowing of the ureter lumen. It will result in the obstruction to the fluid flow in the ureter from kidney to bladder, giving rise to the accumulation of the fluid in the kidney. This is also known as hydronephrosis, in which the kidney swells up, resulting in flank pain and abdominal discomfort. If left untreated for prolonged period, the condition may lead to renal failure and even death in severe cases.⁵

There are two types of causes for ureteric stricture and they can be classified as either intrinsic or extrinsic. Intrinsic factors include scarring due to iatrogenic injuries, passage of kidney stones or infections, whereas extrinsic factors include cancer and compression of ureters, for instance by neighboring organs.⁶ For this thesis, only scarring of the ureter, also understood as the result of an excessive build up of collagen due to iatrogenic injuries, will be addressed.

2.1.3 Current Treatment Options and Challenges

Ureteric stricture can be treated by open surgery or minimally invasive methods such as laparoscopic (keyhole) surgery or endoureterotomy procedures for the removal of excessive collagen in the tissues. Even though open surgery has brought about much clinical successes, clinicians and patients prefer less invasive methods due to reduced costs, hospitalization stay and recovery time and patient trauma. Common endoureterotomy treatments methods include balloon dilation, cold knife incision, electrocautery and laser therapy.² Even though these methods have their respective success, however, there have been reportedly high incidences of stricture recurrence, with up to 30%.⁷ This meant that there is a need for repeated intervention with added medical expenses, treatment times, inconvenience and pain. The recurrence is mainly due to the inefficiency of current treatments in the complete removal of fibroblasts and the possible injuries that may occur during the treatment itself.^{2, 8}

Since it is a common practice to implant a ureteral stent in the ureter post-treatment for up to 4 - 6 weeks in order to ensure that the urine can be properly drained while the ureter is healing,⁹ it will be potentially beneficial if a stent with therapeutic effect can be implanted in place of a conventional bare stent to remove these residual fibroblasts. This forms the basis for the research work.

2.1.4 Ureteral Stent

Ureteral stents are hollow soft tubes, commonly made of polyurethane, that is implanted in the ureter for the support of the opening of the ureteric lumen to allow proper drainage of urine. It measures at around 25 - 30 cm in length and 5 - 7 French in diameter. It is designed to have J shaped feature at the ends to allow for fixation into kidney and bladder to prevent stent migration (Fig 2.3). Moreover, it is lined with stent holes on the surface for the drainage of urine. Ureteral stents are implanted using an ureteroscope and guide wire through guidance under fluoroscopy.¹⁰

Fig 2.3 Commercially available ureteral stent

The ureteral stent technology has witnessed several years of improvement and transformations. Initially, it began with the choice of material for the base stent. Silicone was first adopted in the 1960s as it is the gold standard for biocompatibility however it was later replaced by polyethylene due to its softness which made railroading it through the guide wire difficult. However, polyethylene also suffered from setbacks as it is too stiff and hence very susceptible to fragmentation. Polyurethane was then later introduced due to its intermediate stiffness between that of silicone and polyethylene. It remains widely used till this day, due in part to its efficiency in drainage of urine and ease of fabrication, however, they can be prone to encrustation and bacteria adhesion resulting in biofilm formation on the stent.¹¹

As a result, the current research actively surrounding ureteral stents have shifted towards coatings and blendings to protect the stent. Furthermore, to ease the placement of stent, hydrophilic polymer such as hydrogel coatings have been applied

on the surface. These products have been marketed however, the hydrogels are not swellable. Other areas of emerging research have shed light on drug-eluting ureteral stents to treat stent-related symptoms such as bacterial infection and pain, as well as biodegradable ureteral stents to remove the need for stent removal.¹²

An ideal stent is thought to possess these qualities: Radio-opaque, affordable, easily manoeuvred, stable after placement, biocompatible, resistant to encrustation and bacterial infection, does not result in irritative symptoms, able to relieve obstruction and maintain long-term flow.¹³ However, no such stents currently existed therefore there is a need for continued effort to make improvements to the ureteral stent technology.

2.2 Emerging Research and Innovation

Selected works of relevance are discussed here. Namely, these topics cover the research effort in improving the permeability of the urothelium as well as the field of drug-eluting stents.

2.2.1 Enhancement of Drug Transport Across the Urothelium

There have been reports about several approaches that can be employed to increase the permeability of the urothelium: (i) Physical and chemical methods, (ii) increasing the residence time of drugs on the target tissues and (iii) bioadhesion.¹⁴

Electromotive drug administration (EMDA) or iontophoresis is a physical method that could be employed to enhance this permeability. It involves the use of small electric current to increase absorption of charged or neutral molecules across the urothelium.¹⁵ The use of "cell penetrating peptide" (CPP) is a chemical approach which can be internalized in all types of cells and help enable the delivery of conjugated biomolecules.¹⁶ Furthermore, topical application of protamine sulfate, chitosan and cyclodextrin are known to disrupt the intracellular tight junctions due to their cationic

nature which can interact with the anionically charged glycosaminoglycan (GAGs) layer.¹⁷ Moreover, prior instillation of dimethyl sulfoxide (DMSO) has also been reported to increase the permeability of chemotherapeutic drugs such as paclitaxel.¹⁸

Moreover, the increase of residence time of drug in contact with the abnormal urothelium is reported to provide continuous doses of therapeutic drugs at the desired site for prolonged period of time in order to achieve enhanced uptake of drugs.¹⁹ The use of a drug depot in the bladder that can achieve sustained release of drugs is also reported to be a better alternative to prolonged catheterized infusion of drugs, from a clinical point of view.²⁰ This concept of prolonged exposure of drugs to enhance permeability has been tested. For instance, it is reported in a study that a drug-eluting balloon was implanted in the porcine ureter. The drug paclitaxel was not detected when the balloon was implanted for 1 hour but following 12 hours of implantation, the drug paclitaxel was detected deep in the smooth muscle layer of the ureteric tissue.²¹ This result demonstrates the importance for the drug to be in close contact apposition with the targeted urothelial tissue so as to permeate through the highly impermeable lining for therapeutic effect. Nevertheless, it is impractical from a clinical point of view to leave a non-hollow balloon within the ureter for such long period of time, as it might lead to blockage and hydronephrosis. In another example, Urogen Pharmaceutical is developing on a thermoreversible hydrogel that releases Mitomycin C to the diseased site, particularly for the treatment of upper tract carcinoma, in place of the current standard of care which may involve the complete removal of the affected kidney. After the treatment, the solidified gel will be washed with cold water to be liquidified before removal. This technology has also highlighted the importance for the increased residence time of drugs at the targeted site for therapeutic efficacy in the ureteric tract. However, the gel is only able to remain visible in the pelvicalyceal system for 4 - 6 hours (using computed tomography imaging and fluoroscopic guidance) and also there is no indication that the gel is still continually releasing the drugs throughout these hours.²² Though interesting, however, to this date, none of these techniques have been in clinical practice.

Bioadhesion between the mucoadhesive polymers and extracellular matrix mucin on the urothelium facilitated by the attractive and repulsive forces at the molecular level,

could help to enhance drug absorption via the use of drug carriers such as microspheres, liposomes and nanoparticles.²³ To add on, the bioadhesive carriers based on polymer such as alginate, fibrinogen and chitosan when loaded with anticancer drugs are found to be effective as post-operative chemotherapy in mice.²⁴ Polymer such as polyethylene glycol is also found to be effective in improving the bioadhesion for enhanced drug transport due to its non-specific interpenetration of its polymer chains with the mucus.²⁵

These research efforts have demonstrated and motivated the use of a drug depot system in the diseased area that can deliver drugs over extended period of time so as to prolong the residence time of the drug with the target tissues to improve permeability and drug uptake into the abnormal urothelium. The use of bioadhesives have been thought to be efficacious in facilitating the drug transfer. As such, the reviews have helped to formulate the solution to the problem of ureteric stricture or other possible urologic disorders, in which, in reiteration, a swellable drug-eluting stent is designed and will be implanted in ureter, that absorbs urine and swells to contact with the diseased site in order to deliver drugs in a sustained and targeted fashion.

2.2.2 Drug-eluting Stents

Drug-eluting stents were first adopted for use in the coronary field to combat restenosis. The first drug-eluting coronary stent (DES) was commercialized in 2003 (CypherTM stent, Cordis Corp, Johnson and Johnson Company, Miami, FL, USA), followed by more marketed products from competitor companies, such as TaxusTM stent (Boston Scientific, Natick, MA, USA).²⁶ This very soon attracted the attention of engineers and clinicians in other fields, such as urology. Boston Scientific has since begun to borrow some of the know-how with drug-eluting devices to the urologic field. For instance, the company was actively developing on a ureteric stents that are able to elute triclosan^{5, 27} and ketorolac²⁸ for the management of stent-related symptoms such as biofilm formation on the stent and pain, respectively.

2.3 Drug Delivery Systems

The delivery of drugs can be tuned accordingly to the needs of the application with different disease states. By choosing the most appropriate delivery systems and kinetics, the most suitable system can be designed to meet the clinical need.

2.3.1 Types of Drug Delivery Systems

There are three types of drug delivery systems, namely: Diffusion-controlled, chemically-controlled and solvent-activated systems.

Firstly, diffusion-controlled systems can either involve a matrix or reservoir system (Fig 2.4). For the matrix (monolithic) system, the drug is either dissolved (monolithic solution) or dispersed (monolithic dispersion) in the polymer matrix. The former case is described as having a low drug loading in which all the drugs were homogeneously distributed in the polymer, whereas the latter case is associated with a high drug loading, where the amount of drug loaded was higher than the saturated limit of the drug in polymer, therefore this will result in some drugs being successfully dissolved whereas the remaining undissolved drugs would exist as drug particles dispersed around the matrix. Drugs at the surface will depart first to the surrounding medium before those within the matrix will begin to diffuse following dissolution. Relevant to this thesis, the previous finding by a member from the same research group, Dr Anthony Ng, has indicated that the solubility of the drug mitomycin C (MMC) in poly-L-lactide-co-caprolactone (PLC) polymer was experimentally determined to be 2.55 mg/cm^3 .²⁹ This means that if the amount of MMC loaded in PLC is to be well higher than this value, the system is likely a monolithic dispersion. In most of the cases, the matrix is usually loaded with drugs above the saturation limit in the polymer.

Conversely, the reservoir system is made up of a core surrounded by a layer of non-biodegradable polymer which the drug is gradually released through. The drug release

is controlled by the interaction between the drug and the polymer. Should the polymer ruptures, there might be the risk for "drug dumping", leading to burst release of drugs.³⁰

Fig 2.4 Schematic diagrams showing matrix and reservoir systems

Secondly, there are two types of chemically-controlled system, namely the pendant chain system and the biodegradable system. In the case of the pendant chain system, the backbone of the polymer is bonded with the drug of interest, in which the bond can be hydrolyzed by the enzymes in the body. The drug release in a biodegradable system relies on the degradation of the polymer encapsulating the drugs and unlike pendant chain system, these drugs are uniformly distributed throughout the polymer and are gradually released when the polymer degrades.³¹

Thirdly, there are two types of solvent-activated systems, namely osmotically controlled system and swelling controlled system. The osmotic system comprises of a device with high drug concentrations loaded within and a fluid from the exterior is driven across a semi-permeable membrane to this drug-loaded region. The buildup of pressure in the region forces the movement of drugs out. For the swelling system, the drugs in the hydrophilic crosslinked polymers are released upon the swelling of the polymer.³²

2.3.2 Drug Release Kinetics and Models

Diffusion is defined as the movement of particles from a region of higher concentration to a region of lower concentration. The mass transport phenomena was described in details in 1855 using the Fick's laws of diffusion, namely the Fick's First and Fick's Second Laws.³³

Fick's First Law is associated with steady-state diffusion in which the drug concentration in the diffusion volume remains constant with time but varies only with the position. In one planar geometry, the diffusion flux, that is the amount of drug particles that move across a particular area per unit time, denoted as J (expressed in $\mu\text{g cm}^{-2} \text{s}^{-1}$), can be described as:

$$J = -D \frac{\partial C}{\partial x} \quad (1)$$

where D is the diffusion coefficient (cm^2/s), C is the position-dependent concentration of drug ($\mu\text{g}/\text{cm}^3$), and x is the position that is perpendicular to the central plane of the film (cm), also understood as the distance measured from the solvent-matrix interface. The negative sign indicates that the diffusion occurs down the concentration gradient.

On the contrary, Fick's Second Law is associated with non-steady-state diffusion in which the drug concentration in the diffusion volume changes with both time and position. In one planar geometry, the diffusion flux is defined as:

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} \quad (2)$$

where C in this case is time and position dependent concentration of drug ($\mu\text{g}/\text{mL}$) and t is time (s).

The equations have been used to generate the basis for the several drug release models over the years, however, only the model relevant to the thesis work shall be discussed.

One of the simplest and most well-known drug release model, the Higuchi model, has been used widely used to elucidate drug releasing from a non-degradable monolithic dispersion system.^{34,35}

Firstly, the Fick's First Law is applied on the assumption where steady-state diffusion is present. The equation denoting the diffusion flux is rearranged as such:

$$R_t = -SD \frac{\partial C}{\partial x} \quad (3)$$

where R_t is the diffusion rate of the drug ($\mu\text{g/s}$), S being the cross-sectional diffusion area (cm^2) and the other components remaining the same.

Then, setting the boundary conditions as $C = C_bK$ (at $x = 0$) and $C = C_s$ (at $x = X(t)$), in which C_b is described as the concentration of drug in the release medium whilst C_s being the saturated concentration of drug in the matrix, the diffusion rate is re-written as: (7)

$$R_t = SD \frac{(C_s - C_bK)}{X(t)} \quad (4)$$

As the diffusion rate of the drug, R_t is also understood as the amount of drug released as a function of time, M_t , this of which is also applied with the following mass balance equation for the determination of $X(t)$ where C_0 is the initial drug concentration in the matrix:

$$R_t = \frac{dM_t}{dt} = \frac{d}{dt} \left\{ [C_0^{-1/2} (C_s + C_b K)] S X(t) \right\} \quad (5)$$

The equation follows a series of integration and can be simplified to be:

$$M_t = S[D(C_s - C_b K) (2C_0 - C_s - C_b K) t]^{1/2} \quad (6)$$

Assuming sink condition is achieved, which is also described as the release medium being significantly less than the saturated concentration of drug in the medium, then C_b is almost equal to zero. Also, assuming that the drug loaded is greater than the saturated limit of the drug in the matrix, where $C_0 \gg C_s$, then the equation can be further simplified to be:

$$M_t = S[2DC_s C_0 t]^{1/2} \quad (7)$$

As such, since M_t is proportional to the square root of time, it is expected that the amount of drug released is dependent on the time, where less drug is eluted with time. When M_t vs t plot is re-plotted with time as $t^{0.5}$, then a linear graph will be resulted. Higuchi model is therefore applicable under the described assumptions and elucidates drug release as having a first-order, predominantly diffusion-controlled characteristic in a non-biodegradable, matrix (monolithic) dispersion system.

2.3.3 Sustained and Controlled Drug Delivery

Sustained drug delivery involves the release of drugs over an extended period of time so that the therapeutic effect can be prolonged. Therefore, it is usually preferred for the treatment of chronic (long-term) conditions. The drug release profile may be linear (zero-order) or parabolic (first-order); The zero-order profile can be achieved with a reservoir-membrane system, while the first-order profile can be achieved with a matrix system.^{36,37}

Zero-order (or linear) profile is crucial only under the following conditions:

- (i) The transport of drug across tissue to the target site is quite rapid
- (ii) The drug of interest is very potent with side effects
- (iii) The drug has low stability hence degradation could be fast
- (iv) The drug clearance from the system is very fast
- (v) The therapeutic window is very narrow

In the opposite instance where the release of drug from the device is much faster and higher in amount compared to the level of drug that can be transported to the target site, then controlled (i.e. zero-order) release is not a necessary element of design. This implies that cheaper and slimmer coatings can be used (as a matrix system) in such cases.

2.4 Materials Selection

This section discusses about the properties and rationalizes the choice of materials for each component of the bilayer swellable drug-eluting stent (BSDES), namely the base stent (polyurethane), drug (mitomycin C), drug-carrier polymer (poly-L-lactide-co-caprolactone) and hydrogel (polyethylene glycol diacrylate).

2.4.1 Ureteral Stent: Polyurethane (PU)

As a reiteration from Section 2.1.4, the ureteral stent is currently commonly made of polyurethane due to the ease of fabrication and intermediate in stiffness between that of silicone and polyethylene, which have been used previously. Silicone was replaced due to its very low modulus or softness and the consequent difficulty to be railroaded through guide wire while polyethylene was replaced due to its high stiffness and susceptibility to fragmentation.¹¹

2.4.2 Drug: Mitomycin C (MMC)

Mitomycin C (MMC) belongs to a family of highly cytotoxic antibiotic, produced by *Streptomyces caespitosus* in the 1950s. It has been approved by FDA in 1974 to treat gastric and pancreatic cancer.³⁸

MMC has the molecular formula $C_{15}H_{18}N_4O_5$ and has molecular mass of 334.13 g/mol. It exists as a violet powder and is readily soluble in water at a saturation point of 0.5 mg/mL.³⁹

MMC exerts its anti-proliferative properties by impairing DNA synthesis, mitosis and protein synthesis and its effect is active at each phase of the cell cycle.⁴⁰ It is first reductively activated to a bis-alkylating agent before forming a MMC-DNA adduct with either intrastrand or interstrand crosslinks. As such, the DNA double helix is unable to unwind and therefore there is no parent strand for the synthesis of a daughter strand as DNA replication is inhibited. It is reported that its median inhibitory concentration for inhibition of HeLa cells can be as low as 0.1 μ g/mL.⁴¹

MMC has been hailed as the standard as a single agent for superficial bladder cancer treatment by local intravesical administration of 20-40 mg in 40 mL sterile water.⁴² However, this requires the inconvenient and expensive weekly hospital visit to receive dosage once per week for 6 weeks in the first round of therapy.⁴³ In addition, MMC administration via percutaneous perfusion to the ureter has also been proven successful to reduce ureteral cancer growth.⁴⁴

Though MMC has never been applied to managing iatrogenic ureteral injuries, MMC administration by submucosal injection has been reported to treat stricture in urethra induced from injuries with low recurrence rate (10% during a 6 month follow up).⁴⁵ Moreover, a low dose MMC administration (10 μ g/mL) by 5 min irrigation to injured urethra was found to be effective in-vivo to treat urethral stricture in rat models.^{40c} These examples motivate and reinforce the use of MMC to treat ureteric stricture recurrence in this thesis.

2.4.3 Drug-carrier Polymer: Poly-L-Lactide-co-Caprolactone (PLC)

Poly-L-lactide-co-caprolactone (PLC) is a biodegradable polymer which is considered for the application as it is biocompatible, does not cause excessive inflammatory and toxic effects, can be sterilized and possesses acceptable storage life.⁴⁶

PLC is a copolymer made of poly-L-lactide (PLLA) and poly-caprolactone (PCL) (Fig 2.5). It has been reported that PLC with 70% L-lactide molar content display an elastomeric thermoplastic behavior due to the multi-block chain multi-structure in which the flexible soft segments alternate with hard crystalline blocks. Their flexibility, high elongation at break values and elevated recovery ratios of the copolymer are highly valued traits which have been applied to several biomedical applications, such as fully biodegradable stents with shape memory, scaffolds for stem cell differentiation to smooth muscle cells, and tissue engineered artificial blood vessels. Furthermore, 70/30 PLC has slow degradation with loss of molecular weight by 3rd month and mass (%) only begins to decrease by 2nd month.⁴⁷ Moreover, 70/30 PLC has a low T_g, at below physiological temperature (19-24 °C) which allows it to soften to rubbery state in the body. Also, it displays elastomeric thermoplastic characteristic which is similar to that of the polyurethane stent material used in this study.⁴⁸ Furthermore, burst release of drugs can be prevented due to the hydrophobicity of PLC.⁴⁹ Hence, 70/30 PLC is a potential candidate material as a drug carrier polymer for the application.

Fig 2.5 Chemical structures of monomer L-lactide (top left), monomer caprolactone (top right) and PLC (bottom)

2.4.4 Hydrogel: Polyethylene Glycol Diacrylate (PEGDA)

Polyethylene glycol diacrylate (PEGDA) is a photo-polymerized synthetic hydrogel (Fig 2.6). PEGDA is selected as the candidate material for several reasons. Firstly, it is bioinert, non-toxic and non-immunogenic, therefore it is safe for implantation. Secondly, its intrinsic resistance to cell attachment prevents the formation of biofilm on the surface. Thirdly, owing to its non pH responsiveness, its swelling is unaffected by the large variations of pH level in urine which ranges from 6.5 – 8.0.^{50,51}

Fig 2.6 Chemical structure of PEGDA

References

1. Hacking, C. and H. Knipe. Ureter. ; Available from: <http://radiopaedia.org/articles/ureter>.
2. Herati, A. S.; Atalla, M. A., Distal Ureteral Strictures. In *Smith's Textbook of Endourology*, Wiley-Blackwell: 2012; pp 472-480.
3. Ureters.; Available from: <http://training.seer.cancer.gov/anatomy/urinary/components/ureters.html>.
4. Zacche, M. M.; Srikrishna, S.; Cardozo, L., Novel targeted bladder drug-delivery systems: a review. *Research and reports in urology* **2015**, 7, 169-78.
5. Elwood, C. N.; Lange, D.; Nadeau, R.; Seney, S.; Summers, K.; Chew, B. H.; Denstedt, J. D.; Cadieux, P. A., Novel in vitro model for studying ureteric stent-induced cell injury. *Bju International* **2010**, 105 (9), 1318-1323.
6. Chew, B.; Paterson, R.; Lange, D., Clinical Uses and Applications of Ureteral Stents. In *Polymers for Vascular and Urogenital Applications*, CRC Press: 2012; pp 129-140.
7. Fasihuddin, Q.; Abel, F.; Hasan, A. T.; Shimali, M., Effectiveness of endoscopic and open surgical management in benign ureteral strictures. *JPMA. The Journal of the Pakistan Medical Association* **2001**, 51 (10), 351-3.
8. Tyritzis, S. I.; Wiklund, N. P., Ureteral strictures revisited...trying to see the light at the end of the tunnel: a comprehensive review. *Journal of endourology* **2015**, 29 (2), 124-36.
9. Frequently Asked Questions about Ureteral Stents 2007; Available from: https://www.med.umich.edu/1libr/urology/umureteral_stents.pdf.
10. Ureteral Stents/FAQ. ; Available from: <http://urology.osu.edu/22746.cfm>
11. Venkatesan, N.; Shroff, S.; Jayachandran, K.; Doble, M., Polymers as Ureteral Stents. *Journal of endourology* **2010**, 24 (2), 191-198.
12. Chew, B. H.; Denstedt, J. D., Technology Insight: novel ureteral stent materials and designs. *Nat Clin Pract Urol* **2004**, 1 (1), 44-48.
13. Denstedt, J. D.; Reid, G.; Sofer, M., Advances in ureteral stent technology. *World Journal of Urology* **2000**, 18 (4), 237-242.
14. Tyagi, P.; Wu, P.-C.; Chancellor, M.; Yoshimura, N.; Huang, L., Recent Advances in Intravesical Drug/Gene Delivery. *Molecular pharmaceutics* **2006**, 3 (4), 369-379.

15. (a) Rosamilia, A.; Dwyer, P. L.; Gibson, J., Electromotive drug administration of lidocaine and dexamethasone followed by cystodistension in women with interstitial cystitis. *International urogynecology journal and pelvic floor dysfunction* **1997**, 8 (3), 142-5; (b) Jewett, M. A.; Valiquette, L.; Sampson, H. A.; Katz, J.; Fradet, Y.; Redelmeier, D. A., Electromotive drug administration of lidocaine as an alternative anesthesia for transurethral surgery. *The Journal of urology* **1999**, 161 (2), 482-5.
16. Schwarze, S. R.; Ho, A.; Vocero-Akbani, A.; Dowdy, S. F., In vivo protein transduction: delivery of a biologically active protein into the mouse. *Science (New York, N.Y.)* **1999**, 285 (5433), 1569-72.
17. (a) Smith, J. M.; Dornish, M.; Wood, E. J., Involvement of protein kinase C in chitosan glutamate-mediated tight junction disruption. *Biomaterials* **2005**, 26 (16), 3269-76; (b) Yang, T.; Hussain, A.; Paulson, J.; Abbruscato, T. J.; Ahsan, F., Cyclodextrins in nasal delivery of low-molecular-weight heparins: in vivo and in vitro studies. *Pharmaceutical research* **2004**, 21 (7), 1127-36; (c) Vemulakonda, V. M.; Somogyi, G. T.; Kiss, S.; Salas, N. A.; Boone, T. B.; Smith, C. P., Inhibitory effect of intravesically applied botulinum toxin A in chronic bladder inflammation. *The Journal of urology* **2005**, 173 (2), 621-4.
18. Chen, D.; Song, D.; Wientjes, M. G.; Au, J. L., Effect of dimethyl sulfoxide on bladder tissue penetration of intravesical paclitaxel. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2003**, 9 (1), 363-9.
19. Frangos, D. N.; Killion, J. J.; Fan, D.; Fishbeck, R.; von Eschenbach, A. C.; Fidler, I. J., The development of liposomes containing interferon alpha for the intravesical therapy of human superficial bladder cancer. *The Journal of urology* **1990**, 143 (6), 1252-6.
20. Jeong, B.; Bae, Y. H.; Kim, S. W., Drug release from biodegradable injectable thermosensitive hydrogel of PEG-PLGA-PEG triblock copolymers. *Journal of controlled release : official journal of the Controlled Release Society* **2000**, 63 (1-2), 155-63.
21. Liourdi, D.; Kallidonis, P.; Kyriazis, I.; Tsamandas, A.; Karnabatidis, D.; Kitrou, P.; Spyroulias, G. A.; Kostopoulou, O. N.; Marousis, K.; Kalpaxis, D. L.; Goumenos, D. S.; Liatsikos, E., Evaluation of the distribution of Paclitaxel by immunohistochemistry and nuclear magnetic resonance spectroscopy after the

application of a drug-eluting balloon in the porcine ureter. *Journal of endourology* **2015**, 29 (5), 580-9.

22. Donin, N. M.; Duarte, S.; Lenis, A. T.; Caliliw, R.; Torres, C.; Smithson, A.; Strauss-Ayali, D.; Agmon-Gerstein, Y.; Malchi, N.; Said, J.; Raman, S. S.; Holden, S.; Pantuck, A.; Beldegrun, A. S.; Chamie, K., Sustained-release Formulation of Mitomycin C to the Upper Urinary Tract Using a Thermosensitive Polymer: A Preclinical Study. *Urology* **99**, 270-277.

23. (a) Ratliff, T. L., Identification of pretreatment agents to enhance adenovirus infection of bladder epithelium. *The Journal of urology* **2005**, 173 (6), 2198-9; (b) Lu, Z.; Yeh, T. K.; Tsai, M.; Au, J. L.; Wientjes, M. G., Paclitaxel-loaded gelatin nanoparticles for intravesical bladder cancer therapy. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2004**, 10 (22), 7677-84; (c) Lee, J. W.; Park, J. H.; Robinson, J. R., Bioadhesive-based dosage forms: the next generation. *Journal of pharmaceutical sciences* **2000**, 89 (7), 850-66.

24. Ozturk, E.; Eroglu, M.; Ozdemir, N.; Denkbaz, E. B., Bioadhesive drug carriers for postoperative chemotherapy in bladder cancer. *Advances in experimental medicine and biology* **2004**, 553, 231-42.

25. Lele, B. S.; Hoffman, A. S., Mucoadhesive drug carriers based on complexes of poly(acrylic acid) and PEGylated drugs having hydrolysable PEG-anhydride-drug linkages. *Journal of controlled release : official journal of the Controlled Release Society* **2000**, 69 (2), 237-48.

26. Htay, T.; Liu, M. W., Drug-Eluting Stent: A Review and Update. *Vascular Health and Risk Management* **2005**, 1 (4), 263-276.

27. (a) Cadieux, P. A.; Chew, B. H.; Knudsen, B. E.; Dejong, K.; Rowe, E.; Reid, G.; Denstedt, J. D., Triclosan loaded ureteral stents decrease proteus mirabilis 296 infection in a rabbit urinary tract infection model. *J Urol* **2006**, 175 (6), 2331-5; (b) Mendez-Probst, C. E.; Goneau, L. W.; MacDonald, K. W.; Nott, L.; Seney, S.; Elwood, C. N.; Lange, D.; Chew, B. H.; Denstedt, J. D.; Cadieux, P. A., The use of triclosan eluting stents effectively reduces ureteral stent symptoms: a prospective randomized trial. *Bju International* **2012**, 110 (5), 749-754.

28. Krambeck, A. E.; Walsh, R. S.; Denstedt, J. D.; Preminger, G. M.; Li, J.; Evans, J. C.; Lingeman, J. E., A novel drug eluting ureteral stent: a prospective, randomized, multicenter clinical trial to evaluate the safety and effectiveness of a ketorolac loaded ureteral stent. *The Journal of urology* **2010**, 183 (3), 1037-42.

29. A. H. Ng.; Studies of Biodegradable Drug-eluting Tracheal Stents. Doctoral Thesis. 2012
30. (a) Langer, R., Biomaterials in Drug Delivery and Tissue Engineering: One Laboratory's Experience. *Accounts of Chemical Research* **2000**, *33* (2), 94-101; (b) Langer, R., New methods of drug delivery. *Science (New York, N.Y.)* **1990**, *249* (4976), 1527-1533; (c) Kost, J.; Langer, R., Controlled release of bioactive agents. *Trends in Biotechnology* **2** (2), 47-51.
31. (a) Leong, K. W.; Langer, R., Polymeric controlled drug delivery. *Advanced Drug Delivery Reviews* **1988**, *1* (3), 199-233; (b) Domb, A.; Amselem, S.; Shah, J.; Maniar, M., Degradable polymers for site-specific drug delivery. *Polymers for Advanced Technologies* **1992**, *3* (6), 279-292.
32. (a) Fan, L.-t.; Singh, S. K., Swelling-Controlled Release. In *Controlled Release: A Quantitative Treatment*, Springer Berlin Heidelberg: Berlin, Heidelberg, 1989; pp 110-156; (b) Theeuwes, F.; Swanson, D.; Wong, P.; Bensen, P.; Place, V.; Heimlich, K.; Kwan, K. C., Elementary osmotic pump for indomethacin. *Journal of pharmaceutical sciences* **1983**, *72* (3), 253-258.
33. Fick, A., V. On liquid diffusion. *Philosophical Magazine* **1855**, *10* (63), 30-39.
34. Kuu, W. Y., Wood, R. W., & Roseman, T. J. (1992). Factors influencing the kinetics of solute release. In A. Kydonieus (Ed.), *Treatise on controlled drug delivery* (pp. 37-154). New York: Marcel Dekker, Inc.
35. (a) Higuchi, T., Rate of release of medicaments from ointment bases containing drugs in suspension. *Journal of pharmaceutical sciences* **1961**, *50* (10), 874-875; (b) Higuchi, T., Mechanism of sustained-action medication, theoretical analysis of rate of release of solid drugs dispersed in solid matrices. *Journal of pharmaceutical sciences* **1963**, *52*, 1145-9.
36. Kuu, W. Y., Wood, R. W., & Roseman, T. J. (1992). Factors influencing the kinetics of solute release. In A. Kydonieus (Ed.), *Treatise on controlled drug delivery* (pp. 37-154). New York: Marcel Dekker, Inc.
37. Chien, Y. W., Controlled release of biologically active agents. *Journal of pharmaceutical sciences* **77** (4), 371.
38. Paz, M. M.; Pritsos, C. A., Chapter Seven - The Molecular Toxicology of Mitomycin C. In *Advances in Molecular Toxicology*, James, C. F., Ed. Elsevier: 2012; Vol. Volume 6, pp 243-299.

39. Dorr RT, Liddil JD. (1995). Stability of mitomycin C in different infusion fluids:
compatibility with heparin and glucocorticosteroids. *J Oncol Pharm Practice*, 1(3), 19–24
40. (a) Tomasz, M., Mitomycin C: small, fast and deadly (but very selective). *Chem Biol* **1995**, **2** (9), 575-9; (b) Hahn, M. S.; Liao, H.; Munoz-Pinto, D.; Qu, X.; Hou, Y.; Grunlan, M. A., Influence of hydrogel mechanical properties and mesh size on vocal fold fibroblast extracellular matrix production and phenotype. *Acta biomaterialia* **2008**, **4** (5), 1161-1171; (c) Liatsikos, E. N.; Karnabatidis, D.; Kagadis, G. C.; Rokkas, K.; Constantinides, C.; Christeas, N.; Flaris, N.; Voudoukis, T.; Scopa, C. D.; Perimenis, P.; Filos, K. S.; Nikiforidis, G. C.; Stolzenburg, J.-U.; Siablis, D., Application of Paclitaxel-Eluting Metal Mesh Stents within the Pig Ureter: An Experimental Study. *European Urology* **2007**, **51** (1), 217-223.
41. Kuroda, Y.; Furuyama, J., Physiological and biochemical studies of effects of mitomycin C on strain HeLa cells in cell culture. *Cancer Res* **1963**, **23**, 682-7.
42. Temenoff, J. S.; Athanasiou, K. A.; LeBaron, R. G.; Mikos, A. G., Effect of poly(ethylene glycol) molecular weight on tensile and swelling properties of oligo(poly(ethylene glycol) fumarate) hydrogels for cartilage tissue engineering. *Journal of biomedical materials research* **2002**, **59** (3), 429-37.
43.
http://www.uhn.ca/docs/HealthInfo/Shared%20Documents/Mitomycin_C_Bladder_Therapy.pdf
44. Amano, T.; Naito, K.; Koshida, K.; Kunimi, K.; Morishita, H.; Hisazumi, H., Percutaneous mitomycin C perfusion of bilateral ureteral carcinoma in situ. *Urol Int* **1993**, **51** (1), 46-8.
45. <http://www.amepc.org/tau/article/view/1251/2429>
46. Suming Li, Michel Vert. Biodegradable polymers : polyesters. E. Mathiowitz. Encyclopedia of Controlled Drug Delivery, John Wiley & Sons, pp.71-93, **1999**
47. Huang, Y.; Wong, Y. S.; Wu, J.; Kong, J. F.; Chan, J. N.; Khanolkar, L.; Rao, D. P.; Boey, F. Y. C.; Venkatraman, S. S., The mechanical behavior and biocompatibility of polymer blends for Patent Ductus Arteriosus (PDA) occlusion device. *Journal of the Mechanical Behavior of Biomedical Materials* **2014**, **36** (0), 143-160.

48. Fernández, J.; Etxeberria, A.; Ugartemendia, J. M.; Petisco, S.; Sarasua, J.-R., Effects of chain microstructures on mechanical behavior and aging of a poly(L-lactide-co--caprolactone) biomedical thermoplastic-elastomer. *Journal of the Mechanical Behavior of Biomedical Materials* **2012**, *12* (0), 29-38.
49. (a) Jelonek, K.; Kasperczyk, J.; Li, S.; Dobrzynski, P.; Janeczek, H.; Jarzabek, B., Novel Poly(L-lactide-co- ϵ -caprolactone) Matrices Obtained with the Use of Zr[Acac]₄ as Nontoxic Initiator for Long-Term Release of Immunosuppressive Drugs. *BioMed Research International* **2013**, *2013*, 11; (b) Gan, C. W.; Chooi, W. H.; Ng, H. C.; Wong, Y. S.; Venkatraman, S. S.; Lim, L. H., Development of a novel biodegradable drug-eluting ventilation tube for chronic otitis media with effusion. *The Laryngoscope* **2013**, *123* (7), 1770-7.
50. (a) Browning, M. B.; Cereceres, S. N.; Luong, P. T.; Cosgriff-Hernandez, E. M., Determination of the in vivo degradation mechanism of PEGDA hydrogels. *Journal of biomedical materials research. Part A* **2014**, *102* (12), 4244-51; (b) Tan, F.; Xu, X.; Deng, T.; Yin, M.; Zhang, X.; Wang, J., Fabrication of positively charged poly(ethylene glycol)-diacrylate hydrogel as a bone tissue engineering scaffold. *Biomedical materials (Bristol, England)* **2012**, *7* (5), 055009.
51. Nall, R. Urine pH Level Test. **2016** February 23; Available from: <http://www.healthline.com/health/urine-ph#Overview1>.

Chapter 3

Experimental Methodology

This chapter describes the experimental methodologies and characterization techniques used to address the hypotheses and objectives of the thesis. It begins with the rationale for the selection of materials, the preparation and fabrication of test samples and characterization of materials to assess the functionality of the device based on the swelling and mechanical properties of PEGDA hydrogel and the surface treatment for the adhesion of the hydrogel onto the stent. Next, the methodologies used to carry out cellular and drug release studies in a static condition are discussed. Furthermore, to better simulate the urine flow condition, a dynamic fluid flow system is customized to address and elucidate the drug release behavior and transportation of drugs into explanted porcine ureteral tissues in an ex-vivo model. Lastly, a pilot in-vivo study was carried out to evaluate the feasibility of the device in its ability to deliver drugs to the targeted site effectively and observe for the presence of any side effects. The principles underlying the synthesis and characterization methods are discussed and the analytical parameters of the methodologies are described in detail.

3.1 Materials

Polyurethane double pig-tailed ureteral stent (Universa® Soft) was purchased from Cook Medical, (Limerick, Ireland). Mitomycin C was purchased from Euroasian Chemicals Pte Ltd (India). 70/30 Poly-L-lactide-co-caprolactone (PLC) (Mw 202 000 g/mol) was purchased from Purac Biochem BV (Gorinchem, The Netherlands). The numbers following the copolymer represent the molar ratio of L-lactide/caprolactone monomers respectively. Poly(ethylene glycol)-diol (PEG-OH), acrolyl chloride, phosphate buffer saline tablets (PBS, pH = 7.4), and Irgacure-2959, also known as 2-hydroxy-1-[4-(hydroxyethoxy)phenyl]-2-methyl-1-propanone, were purchased from Sigma-Aldrich. Primary human bladder stroma fibroblasts (HBdSF), fibroblast basal media, fibroblast growth supplement (FGS) (1% v/v), fetal bovine serum (FBS) (2% v/v) and penicillin/streptomycin (1% v/v) were purchased from Sciencell Research Laboratories (CA, USA). The ultraviolet (UV) lamp (365 nm, 4-5 mW/cm²) was purchased from Vilber Lourmat (France). The customized 3-pin chuck for shear adhesion failure analysis experiment and the customized glass fittings and tubes were purchased from UFO Glass (Singapore). Precellys lysing kit containing CK-28 beads were purchased from Precellys Evolution. SPE tubes (StrataTM- X, 33 µm polymeric reversed phase) (surface modified styrene divinylbenzene) with polymeric sorbent mass = 30 mg/mL were purchased from Phenomenex. Porfiromycin were purchased from Toronto Research Chemicals. The explanted porcine ureters were purchased from a pig abattoir (Primary Industries, Buroh Lane, Singapore).

3.2 Preparation and Fabrication of Bilayer Swellable Drug-eluting Ureteral Stent

The preparation and fabrication of the BSDES sample is discussed here. All base stent samples were cut into stent section of 3-cm in length. The rationale and principles behind the methodologies used for coating the stent and optimizing the parameters for surface treatment are discussed.

3.2.1 Spray-coating

Spray-coating is a technique of applying precise thin film coating onto surfaces. The polymer solution to be applied is atomized into fine droplets by the ultrasonic vibrations of the spray nozzle in the center and then sprayed into a stream of gas by nitrogen gas. The sample to be coated will be connected to the mandrel that moves in forward-backward motion while under rotation at controlled rate. The mixture solution is brought to the nozzle by a syringe pump that pumps it at controlled flow rate onto the material surface.¹

Fig 3.1 Schematic diagram of spray-coating

Spray-coating is chosen as the technique as it is simple and different polymer thickness and drug loading can be easily achieved by simply varying the concentration of the drug and polymer solution, flow rate, transverse rate, rotation speed, ultrasonic power and loop number.

For this experiment, PLC (Purac Biochem BV, Gorinchem, The Netherlands) and MMC (Euroasian Chemicals Pte Ltd, India) of varying concentrations were dissolved in DCM (Sigma-Aldrich, Co.) overnight until a homogeneous solution was obtained. The solution mixtures were spray-coated onto the stent section (2.5 cm) using a spray-coater (Sono-tek, Medicoat I) at a flow rate of 0.05 mL/min, rotation of 100 revolutions per min and at a transverse rate of 0.25 cm/s, coated over 2 cm stent section with 150 loops of coating. The power was kept constant at 1.0 V. After that, the stents were air-dried for 1 day before being transferred into 37°C vacuum oven for

a week. Samples with different polymer thicknesses (20, 50, 120, 240 μm) and different drug loading (2.5, 5, 7.5 % w/w) were prepared and characterized for surface morphology and thickness of coating by using scanning electron microscope (SEM) and Image J software and characterized for drug release by using high performance liquid chromatography (HPLC).

3.2.2 Dip-coating

Dip-coating is a simple technique where the substrate to be coated is immersed into a solution mixture and then withdrawn at controlled speed in a given temperature and atmospheric conditions (Fig 3.2). As the solvent evaporates, a polymer film is left behind.²

Fig 3.2 Schematic diagram of dip-coating

Spray-coating produces stent coating of only a few micron sized thick hence it is difficult to remove the very thin coating for Thermogravimetric Analysis (TGA) to assess the amount of residual solvent after sample drying. The coating needs to be rid of DCM content so the device can be safe since DCM is carcinogenic. Therefore, dip-coating is used to fabricate the coated stent to obtain thicker coating. If the TGA result shows a significant loss of DCM in dip-coated stents after 7 days of drying, it can be inferred that the thinner spray-coated films would contain significantly lower amount of DCM.

PLC (10% w/v) and MMC (5% w/w) was first dissolved in DCM homogenously overnight. Next, 2-cm section of the ureteral stent was dipped into the solution

mixture using a dip-coater (DC-mono, NIMA Technology, England) at a rate of 200 mm/s. 3 dips were performed, with an interval of 10 min pause between each dip. The stent was air-dried for a day before it was transferred into the 37°C vacuum oven to dry for 7 days. The stent coating was later peeled off from the stent surface and analyzed by Thermogravimetric Analysis (TGA) for residual solvent.

3.2.3 Synthesis of Polyethylene Glycol Diacrylate (PEGDA)

Polyethylene glycol diacrylate (PEGDA) is synthesized from the reaction between PEG-OH and acryloyl chloride in an anhydrous condition (Fig 3.3).

Fig 3.3 Synthesis of PEGDA from PEGOH and acryloyl chloride

The lone pair electrons of triethylamine remove the proton of PEG-OH, producing a nucleophilic PEG-O⁻ which attacks the electron-deficient carbon of acryloyl chloride. This allows for the acrylation of the PEG-OH while removing Cl⁻ ion.

For the synthesis of polyethylene glycol diacrylate, the following procedures were carried out.³ First, polyethylene glycol diol (PEG-OH) (molecular weight 8kDa) (Sigma-Aldrich, Co.) was dehydrated by azeotropic distillation of water in toluene (Merck) and dried in 90°C vacuum oven. Next, PEG-OH was acrylated by reacting with distilled acryloyl chloride (Merck, Germany) and distilled triethylamine (for High Performance Liquid Chromatography; Fluka, USA) in anhydrous dichloromethane (Sigma-Aldrich, Co.) at a molar ratio of acryloyl chloride: OH = 2:1. The resultant mixture was next filtered for the removal of triethylamine hydrochloride salt, by precipitation in cold anhydrous diethyl ether and then dried under vacuum at 25°C for 2 days. Lastly, PEGDA powder was further purified via dialysis with distilled water using a dialysis membrane (MWCO = 3.5 kDa) at 4°C for

a day and lyophilized. PEGDA powder was characterized by Proton Nuclear Magnetic Resonance ($^1\text{H-NMR}$).

3.2.4 Synthesis of PEGDA Hydrogel

Polyethylene glycol diacrylate (PEGDA) hydrogel was synthesized using the photocrosslinking method.⁴ First, Irgacure-2959 (Fig 3.4), also known as 2-hydroxy-1-[4-(hydroxyethoxy)phenyl]-2-methyl-1-propanone (Sigma-Aldrich, Co.), is UV-irradiated with a UV lamp (Wavelength = 365 nm, Power = 12W) (Spectra-Teknik, France) to produce highly reactive radicals in the initiation step. Next, a series of propagation steps occurs as radicals attack the carbon-hydrogen bond of the acrylate group at the end groups of chains, to crosslink the network via the formation of carbon-carbon bond between two adjacent neighboring PEGDA polymer chains (Fig 3.5). This is the crosslink point. Additional radical groups that do not participate in further propagation steps are terminated by reacting with each other to form a single bond between two atoms in a molecule.

Fig 3.4 Formation of radicals upon UV irradiation of Irgacure-2959

Fig 3.5 Schematic illustration of the formation of PEGDA hydrogel

The fabrication of PEGDA hydrogel is described: PEGDA powder was dissolved in water to make either 5%, 7.5% or 10% (w/v) solution while the photo-initiator Irgacure-2959 in ethanol was kept constant at 0.1% (w/v). The solution mixture (100 μL) was then added into each well of the 96-wells plate and then crosslinked under an ultraviolet light spectra (Wavelength = 365 nm, Power = 12W) (Spectra-Teknik, France) for 10 min at a 1-cm distance apart. The hydrogels were then washed in distilled water overnight before being dried in 37°C vacuum oven for 7 days (Fig 3.6). The hydrogels were then characterized for gel fraction, equilibrium water content, swelling ratio, and degradation which are assessed through mass loss and rheological analysis. These properties are crucial hydrogel characteristics for optimal functionality of the coated stent device, where a slow degradation and high mechanical strength of the material will be desired as preliminary candidate for the subsequent studies to test its performance to transfer drugs to urothelium while maintaining its stability.

Fig 3.6 Dry PEGDA hydrogel (left) and wet PEGDA hydrogel (right)

3.2.5 Spin-coating

Spin coating is a common technique used to apply thin film onto substrates for industrial and technological applications. The technique is easy to use, fast and can produce very uniform films from few nanometers to microns in thickness. It involves drop-casting a solvent containing the molecules of the material of interest on a substrate under rotation at controlled speed. Most of the solvent will be flung off and airflow will dry the solvent, eventually leaving behind the film on the surface of substrate. The even coating is produced by the surface tension of the solution and the centripetal force due to the rotation.⁵

Spin-coating is used here for the simple and quick preparation of the polymer film of uniform thickness and even surface. The spin-coated film will then later be subjected to different duration of plasma treatment for the optimization of plasma conditions to treat the polymer surface.

For this experiment, PLC (0.6% w/v) was first dissolved in DCM homogenously overnight before the polymer solution (100 μ L) was drop-casted onto glass slide measuring 1×1 cm, using a spin-coater (Sawatec) rotating at 4000 rpm for 30 seconds duration. The coated slides were dried in 37°C vacuum oven for 7 days.

3.2.6 Plasma Treatment

Plasma is the fourth state of matter (others being solid, liquid and gas) which is generated when gas in an enclosed chamber is supplied with high electrical energy. It comprises of high-energy free-moving electrons, molecules, ions, atoms, radicals and ultraviolet light. Since it carries very high amount of energy, it is able to initiate treatment on the surface. Plasma activation of the surface can non-selectively create polysort functional groups containing different polar oxygen-containing species onto otherwise non-polar polymer surface mainly through radical reactions which help to increase wettability and hydrophilicity.⁶ In some cases, surface roughness might also

be induced and this could also result in an enhancement of the wettability of surface chemistries.⁷

Therefore, to augment the adhesion between the hydrophobic poly-L-lactide-co-caprolactone (PLC) coating and hydrophilic polyethylene glycol diacrylate (PEGDA), plasma treatment is utilized.

It is important to note that, even though it has been reported that surface wettability may also be achieved by methods such as hydrolysis (via sodium hydroxide) or aminolysis (via ethylene diamine), to create -OH and -NH groups respectively,⁸ these methods however will likely degrade the drug coated. Therefore, chemical approaches were avoided in this project.

The spin-coated films were treated in a Covance multipurpose plasma system with radio frequency oxygen plasma (Femto Science, South Korea). The samples were positioned in between 2 parallel plate electrodes in the plasma reactor chamber. Air was removed from chamber to create vacuum before the power was turned on. The pressure was at 0.2 Torr at the onset of the plasma discharge. Oxygen flow rate was set to 30 standard centimeters cubic per minute (sccm), power at 100 W and over varying durations: 10 sec, 30 sec, 1 min, 2.5 min and 5 min. Following the treatment, the samples were left in the air for the stabilization of the resulted radicals and functional groups. The surface was then characterized for their wetting properties with Contact Angle (Data Physics) using distilled water. A control was also performed where no plasma was performed. All samples were done in sets of five (n=5).

3.2.7 Customized setup for the Coating of Polyethylene Glycol Diacrylate (PEGDA) Hydrogel onto Stent via Photo-crosslinking

After spray-coating and surface plasma treatment, the stent sample was coated with PEGDA hydrogel using the following method. The stent was placed through a thin rod metal and then encased in a plastic tubing (Fig 3.7). The tubing was injected with PEGDA solution and then subjected to irradiation by Ultraviolet light (UV)

(Wavelength = 365 nm, Power = 12W) (Spectra-Teknik, France) for 10 min. Following this, the tubing was cut off and the coated stent section was dried in the 37°C vacuum oven for 7 days. The stent sample will be characterized with Scanning Electron Microscope (SEM), shear adhesion pull-off test, transwell migration cell work, drug release (in static and dynamic fluid flow conditions), *ex-vivo* transport studies and *in-vivo* feasibility study which will be explained in the subsequent sections.

The hydrogel coating was designed to be longer than the drug coating to mimic the intended design of the working prototype to be utilized in the clinical scenario in which tapered ends (Fig 3.8) were included for the ease of device insertion and removal. Since the tapered hydrogel regions cannot swell sufficiently to contact the urothelium, they do not participate in facilitating drug delivery. Hence, it is not necessary to overlay this tapered hydrogel regions under any drug coatings; The drugs were only coated on the region where the overlaying hydrogel was expected to achieve close apposition with the urothelial tissues for enhanced drug permeation. This design of the working prototype will be consistently applied across the various studies in the thesis, such as in the delamination test, drug release, drug transport and *in-vivo* work.

Fig 3.7 Schematic diagram illustrating the method to coat PEGDA hydrogel onto the stent

Fig 3.8 Schematic diagram showing the intended working prototype with the hydrogel coating designed with tapered ends

3.3 Material Characterization

An array of characterization techniques are used to analyze the samples for their morphology, mechanical, degradation and swelling properties. The rationale and principles of the methods are discussed.

3.3.1 Thermogravimetric Analysis (TGA)

Thermogravimetric analysis (TGA) is a method used to characterize the mass of a material as time and/or temperature changes at a controlled rate in a controlled environment. The sample to analyze will be placed on a weighing pan that is resided in a furnace. The sample is subjected to heating or cooling in which the weight change is recorded. Hence, the equipment is able to detect and quantify the loss of solvent due to evaporation at a given temperature, to determine the decomposition temperature and to determine the amount of additives such as plasticizer.⁹

In this project, TGA is used to quantify the amount of residual solvents left after drying. Specifically, it is used to analyze the residual DCM on the dip-coated stent samples (Refer to Section 3.2.2) and the residual water content of the PEGDA hydrogels after 7 days of drying in the vacuum oven at 37°C.

For the analysis of both DCM and water, Thermogravimetric Analysis (TGA, TA Instrument, Q500) is used. For the analysis of DCM, the temperature was gradually increased at 20°C/min to 75°C and then kept isothermal at 75°C for 30 min. For the analysis of water content, the temperature was gradually increased at 20°C/min to 150°C and then kept isothermal at 150°C for 30 min.

3.3.2 Scanning Electron Microscopy (SEM)

Scanning electron microscope (SEM) is an electron microscope that can generate images of the sample of interest with a focused beam of electrons. When the electrons interact with the atoms in the sample, signals which contain information about the surface morphology and composition are produced. There are mainly five components of SEM, namely: Electron source, lenses, scanning coil, sample chamber and SEM detectors.

Electrons are emitted from the electron gun by thermionic heating and condensed into narrow electron beam to be focused by a series of condenser lens. The scanning coil (or deflection coils) allowed for the beam to scan the sample surface in a raster fashion. Different signals are produced from the sample surface, such as the secondary electron (SE), backscattered electron (BSE) and characteristic X-rays. Secondary electrons are low energy electrons ejected from the k-orbitals of the sample atoms upon interaction with the electron beam. These secondary electrons are emitted at region in very close proximity with the sample surface, generating images of the surface topography with high resolution down to <1 nm. On the other hand, backscattered electrons are electrons reflected from the specimen surface by elastic scattering. Because these electrons are emitted from deeper portions within the sample, the resolution of image is poorer than SE images. However, since the intensity of the BSE signal is closely related to the atomic number of the sample (where the atoms with higher atomic number backscatter more efficiently), BSE images are able to provide compositional information about how different elements in the sample are distributed.¹⁰

SEM is used in this study to analyze the surface morphology and cross-section of the coated stent. Besides, the surface topography will also be assessed after the coatings are subjected to surface treatments, namely plasma treatment and ultraviolet (UV) irradiation and immersion into phosphate buffered saline (pH=7.4) for drug release studies.

Samples to be characterized were first sputtered with gold coating using a gold coater (SPI Module) at 20 mA for 30 sec. The samples were analyzed with SEM (JEOL, JSM 6360, Tokyo, Japan) with accelerating voltage of 5 kV and spot size 55.

3.3.3 Nuclear Magnetic Resonance (NMR)

Nuclear magnetic resonance (NMR) is a spectroscopic technique used to determine the chemical structure of an organic compound. It does so by making use of the magnetic property of certain atomic nuclei. Most commonly, hydrogen nuclei is used, where the term can be described as proton NMR (^1H -NMR). Other atoms include ^{13}C , ^{19}F and ^{31}P . To be NMR active, the atoms cannot have even number of protons and even number of neutrons in the nuclei. If the nuclei has odd number of protons and odd number of neutrons (i.e. ^2H), then they are NMR active with integral spin state ($I = 1$) while those with odd masses (i.e. ^1H) are NMR active with integral spin state ($I = \frac{1}{2}$). For instance, if ^1H is placed in a magnetic field, the nucleus can either align itself *with* the field at low energy ($+\frac{1}{2}$) or *against* the field at high energy ($-\frac{1}{2}$). When radio waves are applied, the nucleus of the atom can absorb the energy to allow itself to flip spin state from low energy state ($+\frac{1}{2}$) to high energy state ($-\frac{1}{2}$). This flipping from one alignment to another is known as resonance.¹¹

NMR is used in this experiment to characterize polyethylene glycol (PEG) and polyethylene glycol diacrylate (PEGDA) (before light activated polymerization was carried out). The samples were dried in 37°C vacuum oven for one week to remove any moisture before characterization. NMR was also used to quantify the amount of acrylation. The final product was dissolved in deuterated water D_2O and characterized using ^1H -NMR (400 MHz Varian).

3.3.4 Measurements of Gel Fraction

In the next few sections, the characterizations of PEGDA hydrogels fabricated will be discussed. First, the gel fraction of the hydrogel is discussed.

A hydrogel comprises of a sol and a gel component, where 'sol' represents the liquid, aqueous and soluble part of the hydrogel and 'gel' represents the insoluble and solid-like component of the hydrogel. Gel fraction is defined as the amount of this 'gel' component of the hydrogel, in which the theoretical and experimental dry masses are compared¹². This can be calculated by the equation shown in (1).¹³

$$\text{Gel Fraction(\%)} = \frac{\text{Experimental Drymass}}{\text{Theoretical Dry mass}} \times 100 \quad (1)$$

PEGDA hydrogels prepared from different concentrations of PEGDA (5, 7.5, 10% w/v) with 0.1% (w/v) Irgacure-2959 were washed overnight in distilled water and then dried in 37°C vacuum overnight for a week. The dry masses of these samples were weighed and recorded as experimental dry mass, which is compared with the theoretical dry mass of the PEGDA hydrogel.

3.3.5 Measurements of Equilibrium Water Content

Equilibrium water content is the amount of water the hydrogel retains in the equilibrium state, that is the state in which it is neither gaining or losing any more water. The equation is described in (2).¹⁴

$$\text{Equilibrium Water Content (\%)} = \frac{\text{Wet mass} - \text{Drymass}}{\text{Wet mass}} \times 100 \quad (2)$$

3.3.6 Measurements of Swelling Ratio

Swelling ratio is the extent of expansion the hydrogel undergoes with respect to the initial dry mass, upon absorption of fluid. With higher gel fraction, there is less free volume for solvent uptake due to the greater degree of crosslinking. This will

mean that there is a lower swelling ratio due to the lower availability of space for solvent to occupy. The swelling ratio can be calculated based on equation (3).¹⁵

$$\text{Swelling Ratio} = \frac{\text{Wet mass} - \text{Drymass}}{\text{Drymass}} \quad (3)$$

3.3.7 Measurements of Mesh Size

This free volume or mesh size of the hydrogel is directly proportional to its swelling ratio and indirectly proportional to its crosslink density. Mesh size (ξ) is defined as the linear distance between two adjacent crosslinking site, modulated by the swelling and de-swelling effects due to its water content (Fig 3.9).¹⁶ Its size is controlled by the crosslinking density where the two share an inversely proportional relationship.

Fig 3.9 A crosslinked hydrogel depicting its mesh size

The mesh size can be calculated based on the Flory-Rehner theories summarized by Venkatraman¹⁷, using the equations (4) - (8). G' is the shear storage modulus (Pa) measured with a rheometer after equilibrium swelling has been attained by Day 3. d is density of the network (For PEG, $d = 1.21 \times 10^3 \text{ kg/m}^3$). R is molar gas constant ($8.314 \text{ m}^2\text{kg s}^{-2}\text{K}^{-1}\text{mol}^{-1}$). T is temperature in Kelvins (298 K). $(r_0^2)^{1/2}$ is the root-mean-square end to end distance of the polymer chain in the unperturbed state. M_c is number average molar mass between crosslinks. M_r is molar mass of the repeating unit (For PEG, $M_r = 44 \text{ g/mol}$). l is the carbon-carbon bond length ($1.54 \times 10^{-10} \text{ m}$). C_n is characteristic ratio (For PEG, $C_n = 4.0$). Q_s is swelling ratio, derived from wet mass (W_2) and dry mass (W_1). P_1 is solvent density (1 g/cm^3) whereas P_2 is polymer density

(1.21 g/cm³). This information will allow for the computation of mesh size (ξ) in equation (8).¹⁸

$$G' = 0.5 \times (dRT/M_c) C_s \quad (4)$$

$$(r_0^2)^{1/2} = l (2M_c/M_r)^{1/2} C_n^{1/2} \quad (5)$$

$$Q_s = Q = (W_{\text{wet}} - W_{\text{dry}}) / W_{\text{dry}} \quad (6)$$

$$V_{2,s} = ((Q_s + 1)(P_2/P_1))^{-1} \quad (7)$$

$$\xi = (V_{2,s})^{-1/3} (r_0^2)^{1/2} \quad (8)$$

The fabricated and dried PEGDA hydrogels were weighed for their dry mass before being re-immersed into 3 mL phosphate buffered saline (pH=7.4) and then incubated at 37°C. At pre-determined time points (n=3), the samples were removed from the solution and then their wet masses recorded before transferred back into the solution. This was conducted over a 28-days study period. G' was obtained from the rheological measurement for the calculation of mesh size.

3.3.8 Mass Loss Measurements of Hydrogel

Hydrogels may be degraded hydrolytically via the breakdown of the ester group by water acting as nucleophiles. If there is extensive breakdown of the crosslink points, the chains may become small enough to leach out of the bulk and result in mass loss of the hydrogel. Otherwise, no significant mass loss may be detected. However, in both cases, it is expected that the mechanical strength of the material will decrease, in which can be characterized by the equipment such as rheometer. Therefore, it is important to carry out both measurements of mass loss and mechanical strength to procure a comprehensive understanding of the degradation profiles of PEGDA hydrogel. The mass loss measurements can be calculated using the equation (9).¹⁹

$$\text{Mass loss (\%)} = \frac{\text{Initial dry mass} - \text{Final dry mass}}{\text{Initial dry mass}} \times 100 \quad (9)$$

It is desired that there is low or no mass loss of the hydrogel for two main reasons. Firstly, this is so that no polymer leaching will likely occur in the ureteric tract to reduce chances for tissue response. Secondly, the material would have relatively high mechanical strength to reduce tendency of hydrogel fragmentation and device failure in long-term stent usage of up to 4-6 weeks indwelling duration.

For this experiment, the fabricated and dried hydrogels were weighed for their initial dry mass before being transferred into 3 mL phosphate buffered saline (pH=7.4) and incubated at 37°C. At pre-determined time points (n=3), the samples were removed from the solution and then dried for a week in the 37°C vacuum oven before the dry masses were weighed again. The change in the initial and final masses were recorded and computed.

3.3.9 Rheological Measurements of Hydrogel

Rheology is the study of the flow and deformation of matter (liquid or soft solid) under the effect of an applied force. Under the influence of an applied force (stress), the material can either undergo reversible or irreversible changes in the shape and size of the body, also termed as ‘deformation’.

Shear stress is used as the force in rheology. It is applied on the material, where the force is perpendicular to the surface. Shear stress (τ) is hence defined as the force (F) over the surface area (A) which the force is applied upon. As a result of the shear stress, the material undergoes deformation, and this change is defined as the shear strain (γ). The rate of this change is defined as the shear strain rate (D).

Rheometer can be employed to measure viscosity (η), that is, the resistance to the flow of fluid (expressed in centipoise, cP). The sample of interest is sandwiched between two plates, and the plate at the top will rotate in a direction, whereas the plate at the bottom will stay motionless (Fig 3.10). The resistance of the fluid to this movement of the top plate will be recorded as the viscosity.

Fig 3.10 Schematic diagram of a rheometer

Some materials are not fluids with only viscous property. These materials with both elastic (all energy added to the material is stored in the material) and viscous (all energy added to the material is dissipated into heat to the surrounding) properties are known as viscoelastic materials. Rheometer has the additional function to determine this product property using the oscillation test mode, in which the top plate does not rotate in a single direction but instead in oscillatory direction. Two sweep tests are essential in this mode: Amplitude sweep and frequency sweep, where the former is used before the latter for measurements. In the amplitude sweep test, the amplitude (shear strain, that is the amount of the rotation by the upper plate) changes while the frequency (rate at which the upper plate rotates) is kept constant. The purpose of amplitude sweep is to determine the linear viscoelastic region (LVE), which is the shear strain at which the material is not yet destroyed. Beyond this strain, the material will be deformed permanently and the rheological measurement will not be reliable.

Next, the frequency sweep is conducted using the shear strain (at LVE) derived from the amplitude sweep measurement. In the sweep, the frequency is varied while the amplitude is kept constant. Frequency sweep is able to provide information about the nature of the material (i.e. long-term stability). This stability can be understood from the comparison between storage (elastic) modulus (G') and loss (viscous) modulus (G''). A viscoelastic material is understood as having more elasticity component than its viscous component when $G' > G''$. This allows us to know that the material has relatively higher strength and stability than material with $G' < G''$.^{20,21}

Since hydrogels such as PEGDA hydrogels are viscoelastic materials, they can be analyzed using the oscillatory test in rheology to derive the storage and loss modulus. The rheological measurements of the hydrogel are able to provide information about the comparative stability and mechanical strength of the hydrogel at different concentrations of PEGDA and Irgacure and at different time points.

High mechanical strength of the hydrogel is desired for the application as the coating for ureteric stent so that the device is able to withstand the various clinical manipulations such as stent insertion and removal without hydrogel fragmenting and risk residing in the ureteric tract. Since the process of stent insertion and removal involves stent moving against the uroepithelial tissues with shear force, the rheological measurement is a suitable technique to characterize the mechanical strength of the hydrogel. Moreover, combining measurements of mass loss and mechanical strength will provide useful information about the degradation of the material.

After synthesis and drying of the PEGDA hydrogels, the samples were placed into 3 mL phosphate buffered saline (pH=7.4) and incubated at 37°C. The hydrogels were removed for rheological analysis at predetermined time points (n=3) over a 28-days study period. The hydrogels analyzed were not re-used again; Different sets of PEGDA hydrogels were fabricated for different time points as the rheology could damage the hydrogels due to shear forces.

The mechanical stability of the PEGDA hydrogels were characterized with a rheometer (Anton Paar Physica, MCR 501) using a 10 mm parallel plate geometry. First, amplitude sweep was performed at varying shear strain (0.5 - 10%), constant frequency (1 Hz) and constant temperature (25°C), to obtain the linear viscoelastic (LVE) region, which is the region with the maximum stress or strain before the polymer begins to break down. A shear strain in this LVE region (0.97%) was selected. The frequency sweep measurement was carried out using the shear strain (0.97%) at varying frequency (0.1 - 5 Hz) to obtain the storage modulus (G') and loss modulus (G''). G' and G'' values at 1 Hz were recorded. Relatively low shear strain and frequency have been applied as it is known that the stented ureter responds by abolishing the peristaltic movement and dilates to accommodate the stent,²² hence it is

likely for the hydrogel to experience low amount of strain from the ureter. The study is a preliminary tool to compare the effects of different PEGDA amount on the hydrogel mechanical properties.

3.3.10 Static Water Contact Angle Measurements

With plasma treatment, surfaces can undergo changes in chemistries and topographies. These changes can be characterized by measurements such as contact angle experiment. Contact angle is the angle formed at the intersection of the solid-liquid interface and liquid-vapor interface. Measurement of water contacts is primarily used in wettability studies, which shows the degree of wetting upon interaction between a solid and liquid. Relatively higher wettability (or high hydrophilicity) corresponds to smaller contact angles (less than 90°) whereas lower wettability (or high hydrophobicity) corresponds to larger contact angles (more than 90°).

Herein for the contact angle experiment, the spin-coated polymer films were subjected to different plasma duration and characterized for the surface wettability by static water contact angle (Data Physics) using sessile drop method, with 6 μL dosing volume and 5 $\mu\text{L/s}$ dosing rate. Five sets of samples were prepared for each conditions.

3.3.11 Measurements of Adhesive Strength between Stent and PEGDA Hydrogel via Pull-off Test

As the static water contact experiments utilized spin-coated films and hence could not mimic the conditions of the actual stent samples, a shear adhesion failure analysis experiment was self-designed and conducted on the actual stent samples to compare the effects of plasma treatment times on interfacial adhesion between the hydrogel and PLC-coated surface (Fig 3.11). It is hypothesized that a longer duration of plasma treatment will impart greater adhesion forces between the PEGDA hydrogel and stent, by increasing the wettability of the polymeric stent surface and surface roughness.²³

Since 10% w/v PEGDA hydrogels were found to have the highest mechanical stability and strength, all samples were coated with 10% w/v PEGDA. Different plasma treatments were chosen: No plasma (control), 10 sec and 5 min treatment (Power = 100 W). The shear adhesion failure analysis test was carried out using the customized procedures described: After the stents were plasma treated (if any), they were placed in the plastic tubing (Diameter = 4.5 mm). A steel rod was placed in the lumen and fixed into the eraser as a support. Some amount of space below the tubing was provided to allow placement between the clamps and a section of the stent was clamped by the customized 3 pin screw clamp. The PEGDA solutions were added into the desired compartment shown in the diagram and then UV cross-linked for 10 min at a distance of 1 cm apart.

Immediately following the hydrogel curing, the shear stresses before adhesive failure occurred, was recorded by TCD110 Series Force Measurement System (Chatillon Force Measurement Products, USA) with a maximum load of 10N and moving upwards at a controlled strain rate of 5 mm/min. The force recorded was the maximum value of force required for adhesive failure and the value was then divided by the interfacial area contacting the hydrogel and stent surface, denoted by $\pi \times 0.167 \text{ cm} \times 2 \text{ cm} = 1.049 \text{ cm}^2 = 1.05 \times 10^{-4} \text{ m}^2$ to derive the force of adhesion between the hydrogel and stent interface in N/m^2 . At least 5 sets of measurements were done for each parameter ($n \geq 5$).

Fig 3.11 Schematic diagram of the shear adhesion failure analysis experiment

3.3.12 Preliminary Tactile Assessment of Long-term Adhesion

Due to the limitation in the shear adhesion pull-off test in evaluating the adhesion forces over long term period, a separate study was carried out over 1 month period to assess the number of days that the hydrogels are able to remain firm and adhered onto the plasma treated PLC surface on the stent samples.

The effect of plasma times (10 s and 5 min) were compared, with 5 sets of samples (n=5) for each group. The samples prepared using 5% w/w MMC and 10% w/v PEGDA were placed into 5 mL of phosphate buffered saline (PBS, pH=7.4) and incubated at 37°C. At pre-determined time points, the samples were removed from the solution and observed for indications of delamination.

This was carried out by clamping the uncoated section of the stent between the fingers of one hand while slowly running the extremities of the thumb and index finger of the other hand along the axis of each sample surface thrice, as indicated in the images below, to evaluate the resistance and delamination, or the lack thereof (Fig 3.12 - 3.13).

Fig 3.12 Coated stent sample to be assessed for long-term adhesion

Fig 3.13 Preliminary tactile assessment, showing the right fingers moving along the stent/hydrogel while the left fingers clamping the stent

3.3.13 Atomic Force Microscopy (AFM)

Studies have shown that the addition of surface roughness can augment the wettability induced by the surface chemistries.^{24,25} This means that the interfacial adhesion between the materials can be further enhanced. Surface roughness at the nanoscale level can be measured by atomic force microscopy (AFM).

Atomic force microscopy (AFM) is a type of scanning probe microscope (SPM) which can be used to analyze the height, friction and magnetism of a surface at the nanoscale. Invented in 1986, it comprises of a cantilever with a sharp tip at the end that scans the surface of interest in a raster fashion. The cantilever gets deflected due to the force between the tip and the sample surface. The deflection is a spring action that is governed by the Hooke's law. The laser beam that strikes the cantilever functions to record these deflections when it is reflected onto the position-sensitive detector which is then translated as a 3-dimensional image of the surface on the computer (Fig 3.14).²⁶

Fig 3.14 Principles of AFM

Changes to the surface topography is typically quantified by the root-mean-square (RMS) roughness (R_q), where Z_i is the current Z value, Z_{ave} is the average Z height within a particular area and N is the number of points on the area.²⁷

$$R_q = \sqrt{\frac{(Z_i - Z_{ave})^2}{N}} \quad (10)$$

AFM is adopted in this study to investigate the effect of plasma treatment on roughness of the sample surface. This is important because plasma modifications have reportedly resulted in greater surface roughness and the roughness can increase interfacial adhesion between layers.²⁸

The surface topography of the plasma-treated spin-coated samples was analyzed using a Nanoscope IIIa Atomic Force Microscope (AFM) (Digital Instruments, Santa Barbara, CA). The images were captured in the tapping mode with the use of monolithic silicon NCH-50 Point Probe (NanoWorld AG, Neuchatel, Switzerland). The scan size was set at $2 \times 2 \mu\text{m}$ and the surface was scanned at the rate of 1 Hz using 256×256 pixels of resolution. Surface roughness measurements (RMS value) and the 3-dimensional topographical images were recorded using Nanoscope image processing software version 5.12r5 (Digital Instruments) (n=3).

3.4 Cellular Studies

This section investigates the effect of drug concentration [MMC] and drug-eluting stent samples on the inhibition of fibroblasts cells. As the application is closely related to urology, human bladder stroma fibroblast cells (HBdSF) are selected as the cells of interest for the study. Two different assay experiments are conducted: Cell proliferation assay and transwell migration assay.

3.4.1 Cell Proliferation Assays

Cell-based assays are frequently used to correlate the effects of drugs on the cell proliferation *in-vitro*. There are mainly four types of cell proliferation assays which can be carried out to conduct different measurements, such as metabolic activity, DNA synthesis, markers associated with cell proliferation and ATP concentration.²⁹

This thesis will focus only on the assay to measure metabolic activity. The metabolic activity of the cells in this study is measured using resazurin reduction assay.

Resazurin reduction assay is a cell proliferation assay (i.e. PrestoBlue®, Alamar Blue® assay). Resazurin can be reduced by the reducing activity of metabolically active cells and gets converted into resorufin of a different color (color change from purplish-blue to pink) (Fig 3.15). The quantity of active cells is usually measured with fluorescence using microplate fluorometer equipped with 560 nm excitation/ 590 nm emission filter, where the fluorescence value is directly proportional to the amount of viable cells. Fluorescence is preferred over absorbance due to higher sensitivity.³⁰

Fig 3.15 Conversion of Resazurin to Resorufin via NADH

The therapeutic range of dosage (between minimum effective concentration and maximum toxic concentration) is the recommended dosage required to produce the most effective pharmacological response without side effects.

The purpose of the cell proliferation assay in this study is to investigate the minimum effective concentration (MEC) and growth inhibitory concentration (GI₅₀) of the drug mitomycin C (MMC) required to inhibit the fibroblast cells in-vitro. MEC is defined as the minimum amount of drug to produce therapeutic effect and GI₅₀ is defined as the drug concentration that triggers 50% of maximal inhibition of cell proliferation. These values are able to provide some useful information about a reasonable range of drug dosage to be tailored with the drug-eluting stent device. PrestoBlue® reagent will be used as the cell assay due to the short incubation time (as low as 10 min) and high sensitivity.

The procedures of the experiment are described as follows: Human bladder stroma fibroblasts (HBdSF) (Passage 8) (Sciencell) were seeded into 96-well plates (TPP) with a seeding density of 3×10^3 cells/well. 200 μL of cell culture media (Sciencell), which comprised of 1% fibroblast growth supplement (FGS), 2% fetal bovine serum (FBS) and 2% penicillin/streptomycin (P/S), were added to each well and the plate was incubated at 37°C / 5% CO_2 for one day before mitomycin C (MMC) of different concentrations (0, 0.005, 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 10 $\mu\text{g/mL}$) were added. To each drug concentration, five sets of samples were used ($n=5$). After 3 days of incubation, the cell culture media was removed and the wells rinsed once with 100 μL Dulbecco's phosphate buffered saline ($1\times$ DPBS, $\text{pH}=7.2$) (Lonza) and the cells in the well analyzed using PrestoBlue® reagent (ThermoFisher). The assay was used in accordance to the manufacturer's protocol, in which 90 μL of cell culture media was added to each well and then 10 μL of the PrestoBlue® reagent was pipetted. After 30 min of incubation at 37°C / 5% CO_2 , 70 μL of the mixture solution was transferred to a black cell plate (Corning) and the cell activity quantified using a multi-well plate reader (Tecan, Infinite ® 200) with 560 nm excitation and 590 nm emission.

3.4.2 Transwell Migration Assays

The purpose of this experiment is to elucidate the feasibility of the coated stents in inhibiting human bladder stroma fibroblasts, especially after the drug layer coatings have been subjected to surface treatment and ultraviolet (UV) irradiation. Moreover, another aim is to determine if the photo-initiator concentration (0.1% w/v) and materials, namely PEGDA hydrogel and PLC are cytotoxic.

The test experiment can be set up according to the procedure described: The test samples were separated with the cells seeded at the bottom of the well by a permeable cell culture insert (8 μm pore size). Drugs eluted from the samples will be small enough to pass through the cell culture insert and interact with the seeded cells. This prevents a direct contact between the sample and the cells which would otherwise cause unwanted mechanical cell injury.

For this experiment, human bladder stroma fibroblasts (HBdSF) (Passage 9) were seeded into 24-well plates with a seeding density of 18×10^3 cells/well. 1 mL of cell culture media was added to each well and incubated at 37°C / 5% CO₂ for one day. The well was then rinsed once with 100 µL Dulbecco's phosphate buffered saline (1× DPBS, pH=7.2) before the test samples were added.

The coated stent samples (n=4) were fabricated using the protocols described in Section 3.2.7, with 5% (w/v) MMC coated over 2 cm on a 2.5-cm stent using 150 loops at 0.05 mL/min and 10% (w/v) PEGDA irradiated with 365 nm UV light (10 min) using 0.1% (w/v) Irgacure-2959 as the photo-initiator. The samples were dried and then sterilized using ethylene oxide (ETO) gas before use. Controls were also set up to investigate the feasibility of the coated stent samples in inhibiting fibroblasts and to demonstrate that the PLC and PEGDA materials used on the stent are not cytotoxic to the cells.

The stent samples were then individually placed into the 24-well plates (TPP) with cells attached at the bottom of the well. The samples were submerged completely in the cell culture media (2.2 mL) but without any direct contact with the cells by using permeable cell culture insert (8 µm pore size) (BD Biosciences). The cells were incubated at 37°C / 5% CO₂ for the next three days. The cells in the well were analyzed using PrestoBlue® reagent in which 900 µL of cell culture media was added to each well and then 100 µL of the PrestoBlue® reagent was pipetted. After 30 min of incubation at 37°C / 5% CO₂, 70 µL of the mixture solution was transferred to a black cell plate and the cell activity quantified using a multi-well plate reader (Tecan, Infinite ® 200) with 560 nm excitation and 590 nm emission.

S/N	Descriptions
A	Cell culture media without MMC
B	Cell culture media with 0.5 µg/mL MMC
C	Stent coated with PLC and 10% PEGDA (no MMC is added)
D	Stent coated with 5% MMC-loaded PLC and 10% PEGDA

Table 3.1 Different test conditions for transwell migration assay

3.5 Characterization of Drug Release in a Static Condition

This section discusses the drug release rates and kinetics of the coated stents in a static condition. This is to provide information about the effects of polymer thickness, drug loading, surface treatments, hydrogel and radio-opaque agents, in order to tailor a reasonable range of release rates and amount. Moreover, this section elucidates the mechanism of the drug release and evaluates the compatibility of the drug delivery depot for the treatment of the disease state. Release of the drugs in the static condition is measured using high performance liquid chromatography (HPLC).

3.5.1 High-Performance Liquid Chromatography (HPLC)

High performance liquid chromatography (HPLC) is an analytical technique to separate, identify and quantify chemicals in a mixture, based on solubility and polarity. It comprises a liquid phase (also known as mobile phase) and stationary phase. The sample of interest will be applied to one end of the HPLC column and gets carried along the stationary phase by the mobile phase, until it gets eluted from the other end. The time taken for the sample to be eluted is termed as the 'retention time' and is dependent on the affinity with the stationary phase. The more extensive this interaction, the longer the retention time. Most commonly, a reversed phase HPLC is used, in which the stationary phase is hydrophobic (water-hating or non-polar). This means that hydrophilic (water-loving or polar) samples which would not have a strong affinity with the stationary phase, will be eluted earlier than hydrophobic samples. Because polar samples have weaker interaction with the 'oil' stationary phase, they are eluted before the non-polar samples.³¹

Apart from HPLC, there are other separation methods, such as gel permeation chromatography and ion exchange chromatography which separates chemicals based on size and shape as well as charge and polarity, respectively. In this experiment, only mitomycin C released from the stent device will be measured in the solvent, hence HPLC is used.

Effects of (i) polymer thickness, (ii) drug loading and (iii) PEGDA hydrogel were investigated.

- (i) Different polymer thicknesses were prepared: 50, 120, 240 μm
- (ii) Different drug (MMC) loadings were prepared: 2.5, 5, 7.5 % (w/w)
- (iii) Presence of 10% (w/v) PEGDA coating

For all test samples, they were placed into a snap-cap bottle containing 5 mL phosphate buffered saline (PBS, pH=7.4) and then incubated at 37°C. At pre-determined time points (n=3), the solution (1 mL) was removed from the bottle and then filtered into HPLC vial using 0.22 μm syringe filter (Sartorius AG). The PBS solutions were replenished weekly and the cumulative drug released (%) quantified by HPLC.

The HPLC method is described as follows: The amount of MMC released from the stent samples was quantified using a reversed phase high-performance liquid chromatography (Agilent 1200 Series) equipped with a 250 mm $\times$ 4.6 mm reversed-phase C₁₈ column (Agilent Technologies, CA, USA) with a pore size of 5 μm . The mobile phase was prepared using a solution mixture of distilled water and HPLC grade methanol (Tedia, OH, USA) in the ratio of 3:1 with the addition of 0.5% (v/v) acetic acid (Sigma-Aldrich, Co.) and 0.02 M ammonium acetate (Tedia, OH, USA).³² This mixture was then filtered using 0.22 μm membrane filter (Durapore). Samples (50 μL) were injected into the column and eluted at a flow rate of 1 mL/min (148-149 bar). Elution was run for 15 min for each sample and the mitomycin C (MMC) peak was detected by 365 nm UV lamp³³ with retention time (R_t) = 12 min. All samples were collected at pre-determined time points, in triplicates (n=3). The cumulative drug released can be calculated using the following equation:

$$\text{Cumulative drug release (\%)} = \frac{\text{Concentration of drug } \left(\frac{\mu\text{g}}{\text{mL}}\right) \times \text{Volume of PBS in container (mL)}}{\text{Mass change } (\mu\text{g}) \times \text{Concentration of MMC in PLC } \left(\frac{\text{w}}{\text{v}}\right)} \times 100 \quad (11)$$

Briefly, the mass change before and after spray-coating of the sample accounted for the amount of PLC and drug added to the stent. Amount of drug was calculated by multiplying this change in mass with the percentage of the drug in the stock solution prepared (denominator). Total drug released at the specific time point was calculated

by multiplying the concentration of drug measured by HPLC with the volume of PBS in which the samples were kept under incubation (numerator). A division between the numerator and denominator gave rise to the cumulative drug release (%) as indicated in equation (11).

For the calibration standards, 0.1 mg/mL MMC was dissolved in PBS (pH=7.4) before serially half-diluted 4 times using PBS (lowest down to 6.5 µg/ml) and then characterized using HPLC. The calibration curve with R^2 value was reported in the Appendices section (4i).

3.5.2 Loading Efficiency of MMC onto Stent

The stents were spray-coated to attain different drug concentrations, namely 2.5%, 5%, and 7.5% (w/w) MMC. After the stents were dried for 7 days to remove the residual dichloromethane, they were analyzed for the initial total drug amount loaded experimentally. This experimental amount was compared with the theoretical amount of drug loaded to obtain the loading efficiency (%) of the drug coated.

The theoretical amount of drug loaded can be calculated using the following:

$$\text{Theoretical loading } (\mu\text{g}) = \text{Mass of coating } (\mu\text{g}) \times \text{Concentration of MMC in PLC } (\% \text{ w/v}) \quad (12)$$

The experimental amount of drug loaded can be calculated using the following:

$$\text{Experimental loading } (\mu\text{g}) = \text{Concentration of drug } (\mu\text{g/mL}) \times \text{Volume of DCM (mL)} \quad (13)$$

The loading efficiency (%) can be calculated using the following:

$$\text{Loading efficiency } (\%) = \frac{\text{Experimental loading } (\mu\text{g})}{\text{Theoretical loading } (\mu\text{g})} \times 100 \quad (14)$$

The drug extraction procedures were carried out as follows: The stent samples of different drug concentrations (n=3 for each) were placed in snap-cap vials containing dichloromethane (DCM) (5 mL) for 30 min to dissolve away the coatings of PLC and MMC. The mixture solution was then centrifuged at 5000 rpm for 10 min to remove

any PLC material before the drug in DCM was analyzed using High Performance Liquid Chromatography (HPLC).

Calibration standards were prepared using the same method by dissolving a series of concentrations of MMC in dichloromethane, starting from 50 $\mu\text{g/mL}$ serially diluted in half for 4 times using DCM (lowest down to 3.125 $\mu\text{g/mL}$). The standards were each subjected to centrifugation and then characterized using HPLC. The calibration curve with R^2 value was reported in the Appendices section (4ii).

3.5.3 Stability of MMC

MMC (0.05 mg/mL) was evaluated for its stability in phosphate buffered saline (PBS, pH = 7.4) at 37°C over 28 days period. At pre-determined time points (n=3), 1 mL of solution was removed and then tested with HPLC.

3.6 Design of Customized Setups for the Simulation of Dynamic Fluid Flow Condition

This section will address the question of whether the swelling effect of PEGDA hydrogel will enhance the delivery of drugs into the ureteric tissues in a dynamic fluid flow condition. Here, the customized experimental setups are described to investigate the effect of PEGDA hydrogel on the in-vitro drug release from stent and ex-vivo drug transport into explanted porcine urothelial tissues. Two slightly different setups were designed and created for the release and transport studies to simulate the performance of the stent device in an ureteric condition.

3.6.1 In-vitro Drug Release

This study aims to elucidate the effect of PEGDA hydrogel on the drug release kinetics in a simulated dynamic urine flow condition. It is hypothesized that the hydrogel will increase the amount of drug released as the drug coating section is perpetually surrounded by an aqueous environment (hydrogel in this case). This is in contrast with a stent coated with drug but not hydrogel, in which the drug coating is only in contact with the fluid intermittently in a urine flow environment.

The experiment was set up to mimic as much as possible an adult human physiological urinary system (Fig 3.16 - 3.17): The coated stent of interest was placed in a customized glass tubing (Internal diameter = 4 mm; Outer diameter = 6 mm; Length = 22 cm) (UFO Glass) and a surgical suture line was used to keep the stent positioned in the middle section of the glass tube. Phosphate buffered saline (PBS, pH=7.4, 37°C) was allowed to run through the glass tube via silicone tubing (Cole-Parmer, USA) at a flow rate of 0.5 mL/min³⁴ with the aid of a peristaltic pump. The glass tubing was connected to a customized glass (UFO Glass) shaped like an adult human kidney (Width = 3.5 cm; Height = 5 cm). PBS would collect to a certain amount in the glass before it flowed down the glass tube and the fluid was collected in a container. At pre-determined time points (n=3), the volume of drug-containing PBS would be recorded and 1 mL of the volume analyzed for drug quantity using liquid chromatography mass spectroscopy (LCMS). The test sample comprised of a 3-cm 5-Fr stent section coated with 1-cm 5% (w/v) MMC and 2-cm 10% (w/v) PEGDA hydrogel, while the control sample comprised only of the stent section coated with 1-cm 5% (w/v) MMC. The experiment was carried out over 7-days period for each sample.

For the calibration standards, 1000 ng/mL MMC was dissolved in PBS (pH=7.4) before serially half-diluted 15 times (lowest down to around 0.1 ng/mL) and then characterized using LCMS. The calibration curve with R^2 value was reported in the Appendices section (4iii). With the known concentrations of drug (x) giving rise to the respective peak areas (y), the calibration curve generated a gradient, m in the equation

$y = mx$. The drug amount collected at predetermined time point was analyzed by using the formula: $x = y/m$.

Fig 3.16 Schematic illustration of *in-vitro* dynamic fluid flow model

Fig 3.17 Actual illustration of *in-vitro* dynamic fluid flow model

3.6.2 Ex-vivo Drug Transport

This study aims to elucidate the effect of PEGDA hydrogel on the drug transport into explanted porcine ureteric tissues in a dynamic urine flow condition. It is hypothesized that the hydrogel will enhance the amount of drugs delivered into the ureteric tissues by ensuring that the drugs can be transported into tissues before they are washed away by the fluid.

The experiment is set up according to the protocols herein described: A similar setup was designed like the *in-vitro* dynamic fluid flow model, except that the glass tube was replaced with explanted porcine ureteral tissues (Fig 3.18 - 3.19). The adult porcine ureteral tissues (*Sus scrofa*, Internal diameter = 4 mm) were collected from a local pig abattoir (Primary Industries, Buroh Lane, Singapore) and the fat tissues removed in the laboratory. The ureters were stored in phosphate buffered saline (PBS, pH=7.4) containing tissue preservatives comprising 10% (v/v) dimethyl sulfoxide (DMSO) and 0.05% (w/v) sodium azide, and then frozen at -20°C until use. The experimental setup was placed in a customized sealed acrylic humidifying chamber (Width = 30 cm; Length = 45 cm; Height = 75 cm) containing a dish of distilled water, to allow the humidity to be constant at 70%. This control of humidity would allow the ureters to be kept moist during the experiment which would last 3 days for each sample. The test and control samples to be evaluated were prepared to the same conditions similar to the descriptions in Section 3.7.1. At pre-determined time points (n=5), the ureters were removed and drugs extracted using solid phase extraction and then quantified using liquid chromatography mass spectroscopy (LCMS).

A calibration standard for the quantification of MMC in porcine ureteric tissues was performed using the described method: 8 pieces of 50 ± 5 mg of porcine tissue sections were cut out using a surgical scissor and then each placed in a lysing kit with six hard tissue homogenizing CK-28 beads (Precellys). 1 mL of 90% water/10% methanol was added to each kit and then 10 μ L of MMC dissolved in acetonitrile was spiked to the solution in respective kits to attain MMC amounts of: 0, 2.14, 4.88, 19.5, 78.1, 312, 1250 and 5000 ng etc. Also, 10 μ L of Porfiromycin (PFM) (Toronto

Research Chemicals) was spiked to each kit as the internal standard (IS) in the same amount of 200 ng. Porfiromycin (PFM) is used as an internal standard of Mitomycin C (MMC), to ensure higher reliability of the result since solid phase extraction could potentially lead to sample loss with batch-to-batch variations in sample recovery. Next, the tissues were thoroughly vortex and then homogenized at 10,000 rpm for 6 cycles (10 s per cycle with 10 s pause) using a tissue homogenizer (Precellys Evolution). After that, the samples were centrifuged at 13,000 rpm for 5 min and the supernatant collected. The supernatants were added with 0.2 M zinc sulphate (ZnSO_4) (Sigma Aldrich) and then centrifuged again at 13,000 rpm for 10 min. The resultant supernatant will be further purified with solid phase extraction (SPE) for analysis and concentration of drugs measured using LCMS.

The calibration curve using (area under curve for MMC/ area under curve for PFM) was plotted against (MMC concentrations/ PFM concentrations) as shown in equation (15). This generated a curve with gradient, m with R^2 value. This curve was reported in the Appendices section (4iv).

$$\frac{\text{Area under curve for MMC}}{\text{Area under curve for PFM}} = \frac{[\text{Amount of MMC}]}{[\text{Amount of PFM}]} \times m \quad (15)$$

Fig 3.18 Schematic illustration of *ex-vivo* dynamic fluid flow model

Fig 3.19 Actual illustration of *ex-vivo* dynamic fluid flow model

Following the procedures of the *ex-vivo* drug transport, 50 ± 5 mg of the porcine ureteral tissues that contacted with the drug-eluting stent section were weighed and cut out. The tissue sample was spiked with 10 μL of porfiromycin (100 ng/mL) (Toronto Research Chemicals). The tissue was then left undisturbed for 15 min for PFM to be absorbed thoroughly. The tissue was then processed and analyzed in the same ways as how the calibration standards were made, as described in the above-mentioned paragraph.

For the analysis of the drug amount analyzed, the equation (15) was arranged to give equation (16) as shown below. Since m is derived from the calibration curve and the amount of PFM added (200 ng) was known, so the amount of MMC can be calculated via:

$$[\text{Amount of MMC}] = \frac{\text{Area under curve for MMC}}{\text{Area under curve for PFM}} \times \frac{[\text{Amount of PFM}]}{m} \quad (16)$$

3.6.3 Solid Phase Extraction (SPE)

Solid phase extraction (SPE) is a simple, rapid and selective sample preparation method used to extract, concentrate or purify samples for analysis. For instance,

samples from urine, blood and tissues may be extracted for identification and quantification. Similar to high performance liquid chromatography (HPLC), SPE relies on the affinity between the solutes in a liquid mobile phase and the stationary phase. As the solute passes through the stationary phase, the different components in the solute are separated and the desired analyte of interest is retained in the column before they are eluted as eluent for analysis. There are typically 4 types of SPE, namely reversed phase (polar liquid phase and non-polar modified solid phase), normal phase (non-polar liquid phase and polar modified solid phase), ion exchange (electrostatic attraction of a charged group on compound to the charged group on surface of sorbent) and adsorption (interactions of compounds with unmodified materials). Compared to liquid/liquid extraction, SPE is much more efficient as it can produce quantitative extractions, is fast and can be automated.³⁵ SPE follows a five-steps process, which involves: (1) Selecting an appropriate SPE tube, (2) conditioning of the tube, (3) adding the samples, (4) washing of packing and (5) eluting of the compound of interest.

Following the extraction of the drug from the bulk of ureteric tissues, the sample matrix was further purified using solid phase extraction. For this experiment, 1 mL SPE tube, StrataTM- X, 33 μ m polymeric reversed phase (surface modified styrene divinyl benzene), polymeric sorbent mass = 30 mg/mL (Phenomenex), was used because the drug mitomycin C (MMC) is more soluble in water-miscible organic solvents such as acetonitrile, compared to water.

The SPE tubes were first connected to a vacuum manifold which can allow for the control of solvent flow rate. Next, the tubes were conditioned using methanol (2 mL), followed by ultrapure water (2 mL). After that, 1 mL of the samples were added to the packing and allowed to elute slowly (< 1 mL/min). A series of washing steps were performed in the order: Water (1 mL) X 2, 90% water/10% methanol (1 mL) X 1, and 70% water/30% methanol (0.2 mL) X 1. The samples were then allowed to dry with nitrogen for 5 min. Finally, the analyte retained on the packing was eluted twice using 70% water/30% acetonitrile (0.5 mL) at a controlled flow rate (< 1 mL/min). The drug extracted was then quantified using liquid chromatography mass spectrometry (LCMS).

3.6.4 Liquid Chromatography Mass Spectroscopy (LCMS)

Liquid chromatography mass spectroscopy (LCMS) is an analytical technique which comprises of a high performance liquid chromatography (HPLC) component and a mass spectrometry (MS) component. The HPLC component separates the analytes while the MS component detects the charged species.

The separation process of LCMS follows the description of HPLC. For the MS component, the separated analyte eluted from the column will be sprayed into the Atmospheric Pressure Ion Source (API) in which the analyte is converted from liquid ions into gaseous ions, mainly via electrospray ionization (ESI) process while the bulk of the eluent is channeled to the waste. Next, the mass analyzer is used to isolate the ions according to their mass to charge ratio (m/z). Finally, the ions are quantified by the detector..

ESI process utilizes liquid charge separation and ion evaporation technique to produce gaseous ions. Three processes are involved in transferring the ions from HPLC eluent into gas phase. Firstly, the charged droplets are formed at the capillary tip. Next, the droplets undergo desolvation and lastly, gas phase ions are produced from the highly charged droplets.

Mass analyzer such as quadrupole mass analyzer functions to separate ions based on their mass-to-charge ratio (m/z) when they pass along the central axis of the four parallel poles. Voltages are applied to the poles to create an electrostatic field in order to separate the ions. Quadrupole mass analyzer is commonly used due to the low cost and high reproducibility. The ions are eventually directed to the detector where they are transformed into usable signals. This is done by the generation of secondary ions from incident ions, which then get amplified.³⁶ All samples to be analyzed by LCMS follows the same protocol.

The LCMS method is as follows: For the LC method, Acquity UPLC H Class (Waters) was used. Acquity UPLC BEH C18 column (50 mm × 2.1 mm × 1.7 μm)

(Waters) was used and column temperature set to 40°C. The mobile phase comprised of isocratic elution of 90% water and 10% acetonitrile with 0.1% (v/v) acetic acid, at a flow rate of 0.2 mL/min with run time of 5 min. For the MS method, Acquity Xevo TQ-S Micro MS detector (Waters) was used. The electrospray was operated in the positive ionization mode (ESI+) and the MMC and IS were identified in the multiple reaction monitoring (MRM) mode. The following tuning parameters were employed: Capillary voltage (2.80 kV), desolvation temperature (450°C), desolvation gas flow (800 L/h), cone gas flow (70 L/h) and source temperature (150°C). The parent [MMC+H]⁺ ion generated two daughter ions at m/z 214.756 (cone voltage = 6V, collision energy = 28V) and m/z 242.064 (cone voltage = 6V, collision energy = 8V), while the parent [PFM+H]⁺ ion generated two daughter ions at m/z 241.032 (cone voltage = 8V and collision energy = 26V) and m/z 256.057 (cone voltage = 8V and collision energy = 10V). The monitoring ions of MMC and PFM were m/z 242.064 and m/z 256.057, respectively, which were used for the quantification of drug in the tissue samples. For the quantification of MMC and PFM, the daughter ions of each drug in larger amount (m/z 242.064 for MMC and m/z 256.057 for PFM) were selected for their areas under curve while the daughter ions in smaller amount (m/z 214.756 for MMC and m/z 241.032 for PFM) were used qualitatively for the validation of the drug identity.

3.7 In-vivo Pilot Study

A feasibility study was conducted on 1 adult female porcine weighing 34 kg (*Sus scrofa*) in adherence with National Advisory Committee on Laboratory Animal Research (NACLAR) guidelines, with approval from Institutional Animal Care and Use Committee (IACUC). The animal study was carried out at SingHealth Experimental Medicine Centre (SEMC) on Singapore General Hospital (SGH) campus. The purpose of this study aimed to evaluate the feasibility of the device prototype to swell in ureter to co-opt with the urothelium in order to deliver drugs locally to the targeted site, mainly the middle ureter section *in-vivo*. In this study, the coated stent prototype was implanted into the right ureter while the non-stented left ureter was used as control.

As this was a feasibility study, only 1 animal was required. Porcine model was chosen because of the physiological resemblance of porcine ureter with human ureter .

3.7.1 Fabrication of the Working Prototype

The 6 Fr double pig-tailed stent (Cook Medical) was coated with 400 μg MMC (7.5% w/w MMC over 3 cm in the middle), pre-treated with optimized plasma (100W, 5 min) and then a layer of 10% w/v PEGDA hydrogel was coated over 9 cm in the middle section with the use of a plastic mold tubing of 6 mm internal diameter (Fig 3.20). The setup was irradiated with ultraviolet light (365 nm) for 10 min. To facilitate stent insertion and maneuverability, the hydrogel layer was designed to comprise of 2 tapered ends. Care was taken to ensure that no hydrogel was within the hollow stent lumen so that the device can carry out its conventional function to drain urine. Also, the 2 coils at the ends of the stent were undisturbed during device fabrication so that the stent can be properly fixated in the kidney and bladder for prevention of stent migration. The outer diameter of the coated section in the dry state was around 2.8 mm and can swell to 7 - 7.5 mm when fully swollen after 1 day immersion in phosphate buffered saline. A larger diameter of the hydrogel was selected for *in-vivo* setting as compared with the *in-vitro* experiments, due to the consideration that a ureter will commonly respond to stenting by abolishing its peristaltic movements and dilating.³⁴

Fig 3.20 Schematic diagram showing the coated stent prototype

3.7.2 Pre-implantation Procedures

The animal was anesthetized using TKX cocktail (0.05 mL/kg, IM) and atropine sulphate (50 µg/kg, IM). Before the implantation, the animal was administered with analgesics (Tramadol, 2-4 mg/kg, IM) and antibiotics (Synulox, 0.05 mL/kg, IM) once. Intra-operatively, the anesthesia was maintained using 1-3% Isoflurane. Electrocardiogram (ECG), respiration rate, heart rate and oxygen saturation were also monitored and recorded during the operation.

3.7.3 Implantation Procedures

The bladder-ureteral orifice was first located using the ureterscope and intravenous urogram (IVU) was carried out to image the urinary tract by injecting OmnipaqueTM contrast (300 mg I/ mL) via the ear vein. Before device implantation, the coated stent was immersed in OmnipaqueTM contrast (300 mg I/ mL) for 2 min and then briefly in normal saline to promote X-ray visualization and lubricity for ease of stent placement, respectively. This additional step only increased the diameter of the section to approximately 3.2 mm and did not hinder the clinical procedure to insert stent thereafter. The stent placement was performed via the retrograde method, using a 7 Fr catheter and hydrophilic guide wire and cytoscope (Karl Storz). The guide wire was first inserted into the urinary tract and then the device prototype railroaded through the wire before being implanted into position with the aid of a pusher. The wire was withdrawn thereafter and device left in the porcine for a day (24 hours).

3.7.4 Post-study Procedures

By day 1, the IVU was carried out to ascertain whether the hydrogel has contacted with the urothelium. before animal sacrifice. This was done by running the OmnipaqueTM contrast (300 mg I/ mL) down the ureteric tract. If the contrast media accumulates at the site just before the hydrogel coated section without any of the media coming through the gaps, if any, between the ureteric lining and hydrogel surface, it is an indication that the hydrogel has fully contacted with the ureter.

Nevertheless, it is expected that the contrast will eventually go through the stent holes and gets channeled through the hollow lumen into the bladder.

After that, the animal was sacrificed. Euthanasia was performed using Pentobarbital sodium (300 mg/mL) via intravenous route. Laparotomy was performed using thermal ablation to expose the bilateral ureters before the latters were harvested by surgical removal into 3 segments, comprising of proximal, middle and distal sections, each of 6 cm in length.

3.7.5 Feasibility Analysis

The tissue samples were analyzed for presence and quantity of MMC. Blood (10 mL) was also collected into EDTA tubes before device implantation on day 0 and after animal sacrifice on day 1, and analyzed to evaluate any signs of systemic toxicity. It has been reported that the 400 ng/mL MMC in porcine blood is considered as life-threatening to the animal.³⁷ Tissue and blood analysis were performed using solid phase extraction (SPE) techniques and characterized using Liquid Chromatography Mass Spectrometry (LCMS). To evaluate for signs of urine obstruction due to the expanded coated stent in ureter, X-ray images were taken to analyze the shape and size of the kidney calyx. Distension of the calyx would indicate the occurrence of hydronephrosis (backflow of urine).

Calibration standards were carried out on porcine ureteric tissues and blood supplied by Primary Industries, Buroh Lane, Singapore. For the tissues, fats were first removed in the laboratory. The ureters were preserved until use, as described in Section 3.6.2. For the blood, they were stored in EDTA tubes and frozen at -20°C until use.

The calibration standard for the quantification of MMC in porcine ureteric tissues was described in Section 3.6.2. A calibration standard for the quantification of MMC in porcine blood (Primary Industries, Buroh Lane, Singapore) was also performed using the described method: 11 vials containing 1 mL of blood each was spiked with MMC in different concentrations to attain amounts of: 0, 1.95, 3.90, 7.81, 15.6, 31.2, 62.5,

125, 250, 500 and 1000 ng/mL. 10 μ L of Porfiromycin (PFM) (Toronto Research Chemicals) was spiked to each vial as the internal standard (IS) in the same amount to obtain 50 ng/mL. The samples were then centrifuged at 13,000 rpm for 5 min and the supernatant collected, diluted five times with distilled water and purified with SPE. The amount of drugs was measured using LCMS. The calibration curves for MMC extracted from ureter tissues and from blood were reported in the Appendices section (4iv) and (4v). The calibration curve and the analysis of the MMC concentrations were derived using the same method as described earlier in Section 3.6.2, using the equations (15) and (16).

3.8 Statistical Analysis

Quantitative data was reported as mean $\pm$ standard deviation. Two-tailed unpaired student t-Test assuming unequal variances were conducted for multiple independent sample groups and * $p < 0.05$ was considered to be statistically significant. In all the tests, significant difference was determined with a confidence level of 95% (* $p < 0.05$).

References

1. Medicoat BCC for Drug Eluting Balloon Coatings. Available from: <http://www.sono-tek.com/balloon-catheter-coating-system/>
2. H. Schmidt, M. Menning; Wet coating technologies for glass. INM, Institut für Neue Materialien, Saarbrücken, German. Available from: <http://www.solgel.com/articles/Nov00/mennig.htm>
3. Friedrich, J., Atmospheric-Pressure Plasmas. In *The Plasma Chemistry of Polymer Surfaces*, Wiley-VCH Verlag GmbH & Co. KGaA: 2012; pp 303-335.
4. Schild, H. G., Poly(N-isopropylacrylamide): experiment, theory and application. *Progress in Polymer Science* **1992**, 17 (2), 163-249.
5. Spin coating: A Guide to Theory and Techniques. Ossila. Available from: <https://www.ossila.com/pages/spin-coating>

6. Friedrich, J., Bulk, Ablative, and Side Reactions. In *The Plasma Chemistry of Polymer Surfaces*, Wiley-VCH Verlag GmbH & Co. KGaA: 2012; pp 145-195.
7. Influence of surface roughness on contact angle and wettability. Attension Theory Note 7. Biolin Scientific. Available from: http://www.biolinscientific.com/zafepress.php?url=%2Fpdf%2FAttension%2FTheory%20Notes%2FAT_TN_7_roughness.pdf
8. Rudolph, A.; Teske, M.; Illner, S.; Kiefel, V.; Sternberg, K.; Grabow, N.; Wree, A.; Hovakimyan, M., Surface Modification of Biodegradable Polymers towards Better Biocompatibility and Lower Thrombogenicity. *PLOS ONE* **2015**, *10* (12), e0142075.
9. Thermogravimetric Analysis (TGA). PhotoMetrics, Inc. Available from: <http://photometrics.net/thermogravimetric-analysis-tga/>
10. How Scanning Electron Microscopy Works. Nanoscience Instruments. Available from: <http://www.nanoscience.com/technology/sem-technology/how-sem-works/>
11. J.C.Edwards; Principles of NMR. Available from: <http://www.process-nmr.com/nmr1.htm>
12. Okay, O., General Properties of Hydrogels. In *Hydrogel Sensors and Actuators: Engineering and Technology*, Gerlach, G.; Arndt, K.-F., Eds. Springer Berlin Heidelberg: Berlin, Heidelberg, 2010; pp 1-14.
13. Chowdhury, M. N. K.; Ismail, A. F.; Beg, M. D. H.; Hegde, G.; Gohari, R. J., Polyvinyl alcohol/polysaccharide hydrogel graft materials for arsenic and heavy metal removal. *New Journal of Chemistry* **2015**, *39* (7), 5823-5832.
14. Ren, Z.; Zhang, Y.; Li, Y.; Xu, B.; Liu, W., Hydrogen bonded and ionically crosslinked high strength hydrogels exhibiting Ca²⁺-triggered shape memory properties and volume shrinkage for cell detachment. *Journal of Materials Chemistry B* **2015**, *3* (30), 6347-6354.
15. Kumar, P. T. S.; Praveen, G.; Raj, M.; Chennazhi, K. P.; Jayakumar, R., Flexible, micro-porous chitosan-gelatin hydrogel/nanofibrin composite bandages for treating burn wounds. *RSC Advances* **2014**, *4* (110), 65081-65087.
16. Sannino, A.; Demitri, C.; Madaghiele, M., Biodegradable Cellulose-based Hydrogels: Design and Applications. *Materials* **2009**, *2* (2), 353.

17. Venkatraman, S.; Nixon, A.; Highe, A., The deformation behavior of poly(dimethyl siloxane) networks. II. Equilibrium swelling. *Journal of Applied Polymer Science* **1994**, 52 (11), 1619-1627.
18. Cao, Y.; Lee, B. H.; Peled, H. B.; Venkatraman, S. S., Synthesis of stiffness-tunable and cell-responsive Gelatin–poly(ethylene glycol) hydrogel for three-dimensional cell encapsulation. *Journal of Biomedical Materials Research Part A* **2016**, 104 (10), 2401-2411.
19. Metters, A. T.; Bowman, C. N.; Anseth, K. S., A Statistical Kinetic Model for the Bulk Degradation of PLA-b-PEG-b-PLA Hydrogel Networks. *The Journal of Physical Chemistry B* **2000**, 104 (30), 7043-7049.
20. Rheological analysis. Escubed. Available from: <http://88.208.209.19/~escbu/wp-content/uploads/2016/08/AN009-Rheology-oscillation-and-thixotropy-rev-0.pdf>
21. Rheology Theory and Applications. TA Instruments. Available from: <http://www.tainstruments.com/wp-content/uploads/CA-2016-Rheology.pdf>
22. Cummings, L. J.; Waters, S. L.; Wattis, J. A.; Graham, S. J., The effect of ureteric stents on urine flow: reflux. *Journal of mathematical biology* **2004**, 49 (1), 56-82.
23. Wenzel, R. N., Resistance of solid surfaces to wetting by water. *Industrial & Engineering Chemistry* **1936**, 28 (8), 988-994.
24. Influence of surface roughness on contact angle and wettability. Attension Theory Note 7. Biolin Scientific. Available from: http://www.biolinscientific.com/zafepress.php?url=%2Fpdf%2FAttension%2FTheory%20Notes%2FAT_TN_7_roughness.pdf
25. Semal, S.; Blake, T. D.; Geskin, V.; de Ruijter, M. J.; Castelein, G.; De Coninck, J., Influence of Surface Roughness on Wetting Dynamics. *Langmuir* **1999**, 15 (25), 8765-8770.
26. Atomic Force Microscope. EDInformatics. Education for the Information Age. Available from: http://www.edinformatics.com/nanotechnology/atomic_force_microscope.htm
27. Van Vlierberghe, S.; Vanderleyden, E.; Boterberg, V.; Dubruel, P., Gelatin Functionalization of Biomaterial Surfaces: Strategies for Immobilization and Visualization. *Polymers* **2011**, 3 (1), 114.

28. (a) Cvelbar, U.; Pejovnik, S.; Mozetiè, M.; Zalar, A., Increased surface roughness by oxygen plasma treatment of graphite/polymer composite. *Applied Surface Science* **2003**, *210* (3), 255-261; (b) Hasirci, N.; Endogan, T.; Vardar, E.; Kiziltay, A.; Hasirci, V., Effect of oxygen plasma on surface properties and biocompatibility of PLGA films. *Surface and Interface Analysis* **2010**, *42* (6-7), 486-491; (c) Ozgen, O.; Aksoy, E. A.; Hasirci, V.; Hasirci, N., Surface characterization and radical decay studies of oxygen plasma-treated PMMA films. *Surface and Interface Analysis* **2013**, *45* (4), 844-853.
29. C.Smith.; Cell proliferation assays: Methods for measuring dividing cells. Life Sciences Article. 2012. Available from: <http://www.biocompare.com/Editorial-Articles/117892-Cell-Proliferation-Assays/>
30. T. L. Riss et al.; Cell viability assays. 2013. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK144066/?report=reader>
31. E. F. Rossomando.; HPLC in enzymatic analysis. Wiley Interscience. 1998. Available from: https://books.google.com.sg/books?hl=en&lr=&id=wXpELgpHl0gC&oi=fnd&pg=PR3&dq=hplc+in+enzymatic+analysis&ots=IQCKtbpe3N&sig=R19GcCniVmUboHOi5XZoH_jv9MM#v=onepage&q=hplc%20in%20enzymatic%20analysis&f=false
32. Cohen-Naftaly, M.; Friedman, S. L., Current status of novel antifibrotic therapies in patients with chronic liver disease. *Therapeutic advances in gastroenterology* **2011**, *4* (6), 391-417.
33. Cowan, J., Teaching for quality learning at university – By John Biggs & Catherine Tang. *British Journal of Educational Technology* **2012**, *43* (3), E94-E95.
34. Cummings, L. J.; Waters, S. L.; Wattis, J. A. D.; Graham, S. J., The effect of ureteric stents on urine flow: Reflux. *Journal of Mathematical Biology* **2004**, *49* (1), 56-82.
35. Guide to solid phase extraction. Sigma Aldrich. 1998. Available from: <http://www.sigmaaldrich.com/Graphics/Supelco/objects/4600/4538.pdf>
36. Mass Spectroscopy Fundamental LCMS Introduction. Chromacademy e-learning for the analytical chemistry community. Available from: <http://www.ecs.umass.edu/eve/background/methods/chemical/Openlit/Chromacademy%20LCMS%20Intro.pdf>

37. Dalton, J. T.; Wientjes, M. G.; Badalament, R. A.; Drago, J. R.; Au, J. L., Pharmacokinetics of intravesical mitomycin C in superficial bladder cancer patients. *Cancer Res* **1991**, *51* (19), 5144-52.

Chapter 4

Results: Part I

Fabrication and Characterization of Bilayer Swellable Drug-eluting Ureteral Stent

This chapter discusses the fabrication methods of the coated stent samples. This involves spray-coating the first layer of drug-encapsulated polymer, followed by the photo-crosslinking of the PEGDA hydrogel onto the plasma-treated samples. The synthesis and characterization of the PEGDA hydrogel were analyzed and discussed to determine the most compatible PEGDA concentrations for use. Characterizations included equilibrium water content, swelling ratio, degradation and the rheological properties of the hydrogel. Next, different plasma treatment times were characterized and compared for their effectiveness to improve the interfacial adhesion between the hydrophobic polymer and hydrophilic hydrogel layers. These were done by conducting static water contact angle measurements, shear adhesion pull-off tests, long-term adhesion study and atomic force microscopy to derive an understanding of the changes to the surface wettability and surface roughness. The studies successfully evaluated and determined the conditions necessary for fabricating the coated stent that can potentially create stent prototypes that can withstand mechanical manipulation in the clinical setting.

4.1 Introduction

This chapter reports the steps taken to fabricate the bilayer swellable coated stents and the characterization methods adopted. To recollect, the first layer which comprises of the drug encapsulated polymer was coated via spray-coating and the second layer which comprises of the hydrogel was photo-crosslinked using a customized plastic mold.

The hydrogel was synthesized and characterized to obtain profiles of different hydrogel concentrations for their potential to be used clinically without material failure. Surface plasma treatment was used to ensure that the two layers of coatings of very different surface characteristics and wettability were adhered with each other firmly by increasing the hydrophilicity of the PLC layer. Different plasma durations were evaluated to ensure that the hydrogel adhesion was maintained for the common stent indwelling duration of 4 weeks.

4.2 Experimental Methods

MMC-encapsulated PLC was spray-coated using the method described in Section 3.2.1. PEGDA hydrogel was synthesized using the methods in Sections 3.2.3 and 3.2.4. The hydrogel was characterized for their swelling, mechanical and stability properties using the methods described in Sections 3.3.4 to 3.3.9. The hydrogel of desired concentrations was coated onto the stent using the method in Section 3.2.7. Plasma treatment was used to improve the wettability of the PLC layer so that PEGDA can adhere onto it. Different plasma times were evaluated for changes to the surface properties using static water contact angle (Section 3.3.10), shear adhesion pull-off test (Section 3.3.11), 4-weeks observations for signs of hydrogel delamination under static condition (Section 3.3.12) and atomic force microscopy (Section 3.3.13).

4.3 Fabrication and Characterization of MMC Loaded Layer

The stents were successfully spray-coated with the solution mixture of PLC and MMC. Not surprisingly, the higher drug concentrations created layer of more intense purplish color (mitomycin C) (Fig 4.1).

Fig 4.1 Spray-coated stents

4.3.1 Thermogravimetric Analysis (TGA) of MMC-coated Stent (Prepared by Dip-coating)

TGA detected a weight loss of 0.18% for the dip-coated films at around 38 - 40°C, likely due to residual DCM present in the films as the boiling point of DCM is 39.6°C, close to the detected dip in weight shown on TGA spectrum. However, this residual solvent amount is deemed negligible as it is <1% (Fig 4.2). Hence, this demonstrated the drying conditions were sufficiently effective in the removal of the solvent component so that the device can be safe from potential carcinogenicity of dichloromethane.

Fig 4.2 TGA result of drug-coated stent

4.3.2 Surface morphology of MMC-coated Stent

The cross-section images depict the polymer/drug coating over the stent surface (Fig 4.3). The drug coating, regardless of the amount of drug loaded, remains smooth and uniform without any drug agglomeration. This is an important feature to avoid any major burst release of drugs. Also, no visible cracks or signs of PLC coating delaminating from base stent were observed. This was similar to the observations made by X.W. Ng et al who obtained smooth and uniform polymer coating when polycaprolactone (PCL) was spray-coated onto heart stent.¹ Different thicknesses of the PLC coating has been obtained, measuring at 50, 120 and 240 μm (using Image J).

Fig 4.3 Surface morphology and cross-section of coated stent samples

4.3.3 Loading Efficiency of MMC onto Stent

For all the drug concentrations, very high drug loading efficiency at around 90% was achieved with little drug loss (Fig 4.4). This loading efficiency was determined by first dissolving the coated stents into dichloromethane for around 30 min to remove all drugs and coatings, followed by running the samples with high performance liquid chromatography (HPLC). The amount of drugs extracted was compared with the theoretical amount of drugs coated, by the calculation of: Mass change of stent before and after coating $\times$ Drug concentration in stock solution.

Since there was a slight deviation of the obtained experimental loading compared with the theoretical loading, a correction to the drug released (%) was introduced by multiplying the denominator of the equation (11) reiterated below (from Section 3.5.1) by 0.9, to assess the extent of difference resulted.

$$\text{Cumulative drug release (\%)} = \frac{\text{Concentration of drug } \left(\frac{\mu\text{g}}{\text{mL}}\right) \times \text{Volume of PBS in container (mL)}}{\text{Mass change } (\mu\text{g}) \times \text{Concentration of MMC in PLC } \left(\% \frac{\text{w}}{\text{v}}\right)} \times 100$$

Fig 4.4 Loading efficiency of drug in PLC coated on stent

4.4 Synthesis and Characterization of Polyethylene Glycol Diacrylate (PEGDA) Hydrogel Layer

PEDGA was first synthesized from the reaction between PEGOH and acryloyl chloride, and characterized with proton NMR. Following this, the PEGDA hydrogels were cross-linked using photo-crosslinking method and different hydrogels of varying concentrations (5, 7.5 and 10% w/v) were prepared and characterized for their gel fraction, equilibrium water content, swelling ratio, mesh size, mass loss and rheological properties.

4.4.1 Nuclear Magnetic Resonance (NMR) of PEGDA

The characteristic peaks of PEGDA at 4.3 ppm (assigned as 'd') corresponded to protons in the group (-COO-CH₂) present in the PEG backbone and the other peaks at 5.9 - 6.4 ppm (assigned as 'a, b, and c') corresponded to protons in the group (CH₂=CH-COO) (Fig 4.5). Proton NMR (¹H-NMR) confirmed the PEGDA formation calculated (depicted below) to be 80.6% acrylation (Fig 4.6). This shows the successful synthesis of PEGDA from the reaction process.

Fig 4.5 NMR peaks showing formation of PEGDA

Fig 4.6 NMR diagram showing acrylation of 80.6%

To find 'n' value of 8K PEGDA, $\text{CH}_2=\text{CHO}(\text{CH}_2\text{CH}_2\text{O})_n\text{CH}=\text{CH}_2$,

$$8000 = 12+2+12+1+16+n(12\times 2+1\times 4+16)+12+1+12+2$$

$$8000 = 70 + n(44), \text{ hence } 44n = 7930, \text{ and therefore } 'n' = 7930 / 44 = 180.2$$

Degree of acrylation

$$= \text{integral of shifted CH}_2 \text{ at 4.3 ppm} / [\text{integral of total CH}_2\text{CH}_2\text{O} / n]$$

$$= (d/4) / \{[(e+f+g+h)/4] / 180.2\}$$

$$= (1/4) / \{[(1.3891+1.5826+220.9541+1.2898)/4] / 180.2\}$$

$$= 0.25 / [(225.22/4) / 180.2]$$

$$= 0.25 / 0.31 = 0.806 = \underline{\underline{80.6\%}}$$

4.4.2 Thermogravimetric Analysis (TGA) of PEGDA Hydrogel

TGA detected a negligible loss of residual water (0.95%) (Fig 4.7). Hence, this demonstrated the drying conditions were sufficiently effective in the removal of the water solvent component for the accurate mass measurements and characterization of the hydrogel samples.

Fig 4.7 TGA result of PEGDA hydrogels

4.4.3 Gel Fraction of PEGDA Hydrogel

The gel fraction was found to be as high as 80% for all PEGDA concentrations (Fig 4.8). This implies that there is a very high efficiency in the crosslinking process. This is a common observation also made by other groups.² The high cross-linking demonstrated the successful fabrication of the hydrogel for the subsequent characterizations.

Fig 4.8 Gel fraction of PEGDA hydrogels

4.4.4 Equilibrium Water Content of PEGDA Hydrogel

The equilibrium water content of the PEGDA hydrogels followed in the order: 5% > 7.5% > 10% (w/v) (Fig 4.9). The greater water uptake at lower hydrogel concentration is due to the decrease in cross-link density and hence greater distance between the crosslinks, also known as mesh size. With bigger mesh size, water can enter the hydrogel more easily. Nevertheless, for all concentrations of PEGDA, the water content was substantial at >90%. The high amount of water was expected to facilitate in the partitioning, dissolution and diffusion of water-soluble drugs from the polymeric surface, through the hydrogel, to the target tissues. Moreover, due to the

high water content, the hydrogel can provide hydration to the tissues, acting as natural permeation enhancer for the drugs.^{2a}

Fig 4.9 Equilibrium water content of PEGDA hydrogels

4.4.5 Swelling Ratio of PEGDA Hydrogel

The swelling increased along with time and attained equilibrium swelling on Day 3. The swelling ratio of the PEGDA hydrogels, like the equilibrium water content, followed in the order: 5% > 7.5% > 10% (w/v), and they ranged in the swelling amount of 15-30 times (Fig 4.10). The decrease in swelling at higher PEGDA concentrations was due to the increase in the crosslinks which restricted the movement of polymer chains. This relationship between swelling ratio and PEGDA content has been well documented, though other factors that can reduce swelling include the increase of photo-initiator concentration and reducing PEG chain length³. However, in this study, the photo-initiator and PEG molecular weight (Mw) has been kept constant at the minimal amount of 0.1% (w/v) for cyto-compatibility reason and at Mw = 8K as a preliminary starting candidate.

Furthermore, it is also important to note that the swelling ratio for 5% (w/v) PEGDA hydrogel has decreased significantly from Day 21 onwards (* $p < 0.05$). This could be attributed to the degradation of the hydrogel. To confirm this, two experiments would be performed to evaluate the material stability: mass loss measurements and rheological testing.

Fig 4.10 Swelling ratio of PEGDA hydrogels

4.4.6 Mesh Size of PEGDA Hydrogel

The mesh size was greatest for 5%, followed by 7.5% and lowest at 10% (w/v) hydrogel (Fig 4.11). This was confirmed by the computation of the mesh size using equations 4 - 8 described earlier. Moreover, over time, the mesh size across all hydrogel concentrations increased. The phenomenon may be explained by the fact that, as PEGDA degrades hydrolytically, crosslinks between the network chains will break, and hence results in the bigger mesh size.⁴ However, this does not concur with the observed swelling ratio (Fig 4.10), as the extent of swelling either decreased (for 5% w/v) or remained constant (for 7.5% and 10% w/v), rather than increased as mesh size increased. Therefore, we hypothesize that the increase in mesh size may be dominated by the decrease/change in storage modulus rather than the change in

swelling ratio. To confirm this, rheological measurements will be performed to analyze the storage modulus of the hydrogels over time.

To add on, with the mesh size below 1000 nm for all hydrogel concentrations studied, the diffusion of small molecular weight drug, mitomycin C (334 g/mol), is expected to be unhindered and hence the hydrogel is able to carry out its function to deliver drugs to the urothelium.

Fig 4.11 Mesh size of PEGDA hydrogels

4.4.7 Mass Loss of PEGDA Hydrogel

No significant mass losses were recorded for 7.5% and 10% (w/v) PEGDA hydrogels, whereas the mass of 5% (w/v) PEGDA hydrogels were significantly lower at Day 21 compared to Day 7 (* $p < 0.05$) (Fig 4.12). This shows that the mechanical stability of the hydrogel is higher with PEGDA concentrations of 7.5% (w/v) or higher.

The mass loss for 5% (w/v) may be attributed to the breakdown of the PEGDA chains due to the hydrolysis of the ester linkages as water acts as nucleophile to attack the electron-deficient carbon of -COO group, as well as the leaching out of the uncrosslinked PEGDA. Since a one-day washing step with distilled water was already performed (Section 3.2.4) to remove uncrosslinked PEGDA, the mass loss observed were the hydrolyzed PEGDA chains. When compared with the results in Fig 4.10 -

4.11, it is likely that the PEGDA chains for 7.5 - 10% (w/v) hydrogel have only lysed (since mesh size increased along time) but not leached out of the bulk since no mass loss was recorded over the days and swelling ratio remained constant as soon as equilibrium swelling was attained.

Therefore, based on the study, hydrogel concentration was selected at 7.5 - 10% (w/v) concentration as they are more likely to possess greater mechanical moduli. It is important that the hydrogel maintains its structural and mechanical integrity in the ureteric tract to continuously co-opt with the urothelium over the indwelling stenting period without breakage, collapse or delamination.

Fig 4.12 Mass loss measurements of PEGDA hydrogels

4.4.8 Rheological Properties of PEGDA Hydrogel

Rheological results demonstrated that the shear storage modulus of the hydrogel follows in the order: 10% > 7.5% > 5% (w/v) (Fig 4.13). This is because higher cross-link density will result in higher mechanical strength and hence, higher propensity to withstand changes to the mechanical integrity upon stress.

Furthermore, the storage modulus decreases for all hydrogel concentrations over time. Therefore, the increase in mesh size (Fig 4.11) is indeed predominantly due to the decrease in storage modulus rather than the change in swelling ratio. This implies that

crosslinks are being cut over time and not much free chains are being formed for 7.5% and 10% (w/v) hydrogel since their swelling ratios remained fairly constant soon after equilibrium swelling was attained.

Therefore, 10% (w/v) PEGDA hydrogel was selected as the candidate material composition for use, due to its highest potential to withstand mechanical manipulations in the clinical setting.

Fig 4.13 Rheological measurements of PEGDA hydrogels

4.4.9 Coating of PEGDA Hydrogel onto Stent

The coating of the hydrogel onto the stent follows the procedure: The stent was first plasma treated and then placed through a thin rod metal and then encased in a plastic tubing mold. The casing with the stent section was injected with PEGDA solution and then subjected to irradiation by Ultraviolet light (UV) (Wavelength = 365 nm) (Spectra-Teknik, France) for 10 min. Following this, the tubing was cut off and the coated stent (Fig 4.14) was dried in the 37°C vacuum oven for 7 days. The sample was then characterized and studied with scanning electron microscope (SEM) (Fig 4.15), adhesion test (Chapter 4) and drug release under static (in-vitro) (Chapter 5), dynamic fluid flow (in-vitro) conditions (Chapter 6), ex-vivo setting (Chapter 6) and preliminary in-vivo work (Chapter 7).

Fig 4.14 A section of the bilayer coated stent sample

Fig 4.15 Cross-section of the coated stent, depicting the drug and hydrogel coatings

4.5 Surface Plasma Treatment of Stent to Improve Adhesion of PEGDA Hydrogel on PLC-coated Stent

Modification of the surface is important to improve the interfacial adhesion between the hydrophilic hydrogel (PEGDA) and hydrophobic drug-encapsulated polymeric (PLC) surface. Plasma treatment of the surfaces is a widely known technique used to impart wettability to the surface for increased interfacial adhesive forces. It also may have, under prolonged extended exposure, lead to changes in surface roughness. Very few reports can be found on the plasma treatment of PLC surface. Notably, Boonyawan et al studied the effect of NH_3 plasma treatment time on the wettability of PLC for the support of cell biocompatibility and found that the contact angle decreased significantly at 5 min, but did not decrease further upon longer plasma time. Moreover, the study outlined the value of plasma treating PLC is not cytotoxic to cells and may even enhanced cell health.⁵

4.5.1 Static Water Contact Angles of Spin-coated Polymer Films

The following contact angle measurements shows that there is significant reduction in the contact angles, from $90.5 \pm 0.8^\circ$ to $70.6 \pm 0.9^\circ$, when the samples are subjected to 10 seconds (* $p < 0.05$) plasma treatment. This is due to the greater wettability of the surface imparted by the addition of oxygen-containing functional groups onto polymeric surface due to the plasma treatment. However, there is no significant improvement in the contact angles when plasma treatment is prolonged from 10 s to 5 min (** $p > 0.05$). This could be due to the saturation of the oxygen containing functional groups added to the surface. Similar observation with saturation of the functional groups have been made by other groups.⁶ (Fig 4.16)

Since the study was conducted on flat spin-coated film, it could not fully mimic the actual condition of the tubular stent samples. Therefore, subsequently, a shear adhesion pull-off test was carried out to evaluate the interfacial adhesion strength between the drug/polymer and hydrogel layers.

Fig 4.16 Effect of plasma time on contact angles

4.5.2 Force Measurements of Adhesion Strength of PEGDA Hydrogel on the Stent via Pull-off Test

With plasma treatment, it required a significantly greater amount of force to remove the hydrogel from the stent surface, with $9307 \pm 1231 \text{ N/m}^2$ (10 s treatment) and $10356 \pm 1414 \text{ N/m}^2$ (5 min treatment), compared to only $7288 \pm 895 \text{ N/m}^2$ (no treatment) (* $p < 0.05$). However, there was no significant difference in the force required for 10 s and 5 min plasma times (** $p > 0.05$) (Fig 4.17). Hence, plasma treatment has significantly augmented the adhesion of the hydrogel onto the stent, though longer plasma exposure beyond 10 s did not demonstrate further improvement based on both contact angles and adhesion tests.

Fig 4.17 Effect of plasma time on forces of adhesion

4.5.3 Preliminary Tactile Assessment of Long-term Adhesion

The shear adhesion test is limited by its design to assess the adhesion strength in the longer term over span of a common stent indwelling duration for stricture management, that typically takes place for 4 - 6 weeks. Moreover, the hydrogels in

the shear adhesion test were not exposed to the media hence they could not have reached equilibrium swelling state. Therefore, a preliminary tactile assessment was carried out to observe the number of days the hydrogel could remain intact and stayed firmly adhered on the stent samples.

The results demonstrated that 5 min plasma treatment enabled the hydrogel to remain adhered onto the stent for the 4 weeks period, however, when 10 sec plasma was applied, the hydrogel began to delaminate by the first week. This suggested that longer plasma times enhanced the duration of adhesion of hydrogel coating on the stent (Fig 4.18).

However, the observation was not in agreement with the findings in Sections 4.5.1 and 4.5.2 where 10 s and 5 min plasma times yielded similar results with contact angle and shear adhesive force measurement. This might be because the characterization methods might not have been sensitive enough to elucidate the differences in surface characteristics induced by the short and long plasma times.

Since it has been widely reported that plasma treatment may cause polymer etching and lead to more oxidized or rougher surfaces where interfacial adhesion between the surfaces could be increased,⁷ therefore, AFM measurements will be carried out to compare the effects of 10 s and 5 min plasma times on the surface characteristics, such as the extent of oxidation and roughness.

Fig 4.18 Effect of plasma time on long term adhesion of hydrogel on stent samples

4.5.4 Effect of Plasma on Surface Roughness

There was no significant difference in the surface roughness between 0 s and 10 s plasma, with measurements of 7.45 ± 1.84 nm and 6.89 ± 2.18 nm, respectively (* $p > 0.05$). However, 5 min plasma time has created significantly greater roughness of 18.08 ± 4.90 nm (* $p < 0.05$) (Fig 4.19). This could explain for the longer duration of the adhesion observed in the 4-weeks study. The result is consistent with previous works.^{7b, 9}

When comparing the result with previous experiments, contact angle and shear adhesive pull-off tests, the latter used may not be sensitive enough to detect the changes done to the surface at the nanometer level. This is because the wettability may have attained its maximum value with minimum amount of oxygen-containing functional groups on the surface and the longer duration of adherence observed for 5 min plasma treatment may be due to the retention of sufficient oxygenated groups over time, compared to the 10 s plasma exposure.

However, since the polymer surface has roughened due to etching from the 5 min plasma time, a study is later conducted to evaluate the drug release profiles under the effects of plasma modifications, to assess if this could possibly lead to burst release or increase of drug release.

Fig 4.19 Effect of plasma time on surface roughness

4.6 Discussions and Conclusions

The characterization of the different PEGDA concentrations suggested that higher hydrogel concentrations possessed greater mechanical stability with higher storage modulus. However, because of the greater crosslink density, the mesh size decreased and hence swelling ratio also reduced. However, equilibrium water content remained sufficiently high (mitomycin C would likely dissolve in and gets carried to the urothelium) and no degradation via mass loss (not likely to undergo fragmentation of hydrogel) over the 4 weeks study period was recorded for high PEGDA concentrations at 7.5% - 10% (w/v). The reduction in their elastic modulus (hydrogel softening) along time was correlated with the increase in mesh size as crosslink density reduced when some polymer chains began to be hydrolyzed. However, since there was no mass loss, there was likely no polymer leaching from bulk hydrogel.

Nevertheless, even with the increase in mesh size along time, the swelling ratio for 7.5% - 10% (w/v) hydrogel did not increase significantly. This implies that the crosslink density decreases over time for the 7.5% and 10% (w/v) hydrogels and is reflected in a lowered modulus over time. Clearly, such behavior of the hydrogel is practically acceptable, since it means that the hydrogel remains in contact on the urothelium over 4 weeks without exerting undue pressure. Moreover, if significant mass loss occurs, then intimate contact with the urothelium may be lost, and such behavior rules out the use of 5% (w/v) hydrogel.

The characterizations of surface plasma treatments using the different techniques indicated that plasma treatment significantly improved the interfacial adhesion between hydrogel and stent. The contact angle and shear adhesion tests demonstrated that significant reduction in contact angles and increase in delamination forces were observed when plasma treatment was carried out, though there was no difference between 10 s and 5 min plasma treatment time. However, the long-term preliminary tactile assessment and AFM measurements showed that 5 min plasma treatment induced more long-lasting adhesion, as well as more extensive surface roughness, respectively, as compared with no or 10 s plasma treatment. As discussed, the spin-coated films for contact angle measurements might have already been saturated with oxygen content by 10 s plasma treatment hence no further change in contact angle was recorded when treatment was extended to 5 min. The similarity in delamination forces with 10 s and 5 min plasma may be attributed to the lack of sensitivity of the setup in detecting the change and hence the delamination test may only serve as a preliminary validation that the plasma treatment was beneficial to the enhancement of the interfacial adhesion forces.

In conclusion, it is technically feasible to fabricate the BSDS prototype in the lab. 10% (w/v) PEGDA was found to be most compatible for use to potentially withstand mechanical manipulation in the clinical setting and resist material failure, based on the stability and mechanical properties. Moreover, plasma surface treatments (100W, 5 min) was effective in ensuring that the hydrogel remained firm on the stent samples for the required 4 weeks period. This surface improvements involved a combination of changes to wettability and the extent of surface roughness.

References

1. Ng, X. W.; Huang, Y.; Liu, K. L.; Lim, S. G.; Chen, H. H.; Burnett, J. C.; Boey, Y. C. F.; Venkatraman, S. S., In vitro evaluation of cenderitide-eluting stent (CES) I —an anti-restenosis and pro-endothelization approach. *Journal of pharmaceutical sciences* **2014**, *103* (11), 3631-3640.
2. (a) Wong, R. S.; Ashton, M.; Dodou, K., Effect of Crosslinking Agent Concentration on the Properties of Unmedicated Hydrogels. *Pharmaceutics* **2015**, *7* (3), 305-19; (b) Padmavathi, N. C.; Chatterji, P. R., Structural Characteristics and Swelling Behavior of Poly(ethylene glycol) Diacrylate Hydrogels. *Macromolecules* **1996**, *29* (6), 1976-1979.
3. (a) Beamish, J. A.; Zhu, J.; Kottke-Marchant, K.; Marchant, R. E., The Effects of Monoacrylated Poly(Ethylene Glycol) on the Properties of Poly(Ethylene Glycol) Diacrylate Hydrogels Used for Tissue Engineering. *Journal of biomedical materials research. Part A* **2010**, *92* (2), 441-450; (b) Wong, R. S. H.; Ashton, M.; Dodou, K., Effect of Crosslinking Agent Concentration on the Properties of Unmedicated Hydrogels (). *Pharmaceutics* **2015**, *7* (3), 305-319.
4. Hahn, M. S.; Liao, H.; Munoz-Pinto, D.; Qu, X.; Hou, Y.; Grunlan, M. A., Influence of hydrogel mechanical properties and mesh size on vocal fold fibroblast extracellular matrix production and phenotype. *Acta biomaterialia* **2008**, *4* (5), 1161-1171.
5. Daranarong, D.; Techaikool, P.; Intatue, W.; Daengngern, R.; Thomson, K. A.; Molloy, R.; Kungwan, N.; Foster, L. J. R.; Boonyawan, D.; Punyodom, W., Effect of surface modification of poly(l-lactide-co-ε-caprolactone) membranes by low-pressure plasma on support cell biocompatibility. *Surface and Coatings Technology* **2016**, *306*, 328-335.
6. Choi, D. M.; Park, C. K.; Cho, K.; Park, C. E., Adhesion improvement of epoxy resin/polyethylene joints by plasma treatment of polyethylene. *Polymer* **1997**, *38* (25), 6243-6249.
7. (a) Ozgen, O.; Aksoy, E. A.; Hasirci, V.; Hasirci, N., Surface characterization and radical decay studies of oxygen plasma-treated PMMA films. *Surface and Interface Analysis* **2013**, *45* (4), 844-853; (b) Hasirci, N.; Endogan, T.; Vardar, E.; Kiziltay, A.; Hasirci, V., Effect of oxygen plasma on surface properties and

biocompatibility of PLGA films. *Surface and Interface Analysis* **2010**, 42 (6-7), 486-491.

8. Murakami, T.; Kuroda, S.-i.; Osawa, Z., Dynamics of Polymeric Solid Surfaces Treated with Oxygen Plasma: Effect of Aging Media after Plasma Treatment. *Journal of Colloid and Interface Science* **1998**, 202 (1), 37-44.

9. Riau, A. K.; Mondal, D.; Yam, G. H. F.; Setiawan, M.; Liedberg, B.; Venkatraman, S. S.; Mehta, J. S., Surface Modification of PMMA to Improve Adhesion to Corneal Substitutes in a Synthetic Core–Skirt Keratoprosthesis. *ACS Applied Materials & Interfaces* **2015**, 7 (39), 21690-21702.

Chapter 5

Results: Part II

Characterization of the Drug Release Rates in Static Condition for the Inhibition of Human Stromal Bladder Fibroblasts (HBdSF) Proliferation

This chapter discusses the characterization of the drug release from the stent samples in the static condition. Samples of different drug release rates were designed and studied. The effects of polymer thickness, drug loading and PEGDA hydrogel on drug release rates were investigated. The mechanism of the release of drugs was also elucidated. A cellular assay experiment was carried out to determine the feasibility of the coated stent samples in inhibiting human bladder stroma fibroblasts in-vitro. The drug delivery system is evaluated for its compatibility for the treatment of the disease state. The studies successfully demonstrated that tunable amount of drugs could be released and this was above the minimum effective concentration to inhibit the fibroblast proliferation. Moreover, no significant difference in drug release was observed with the additional hydrogel in the static condition.

5.1 Introduction

This chapter reports on the characterization of drug release from the stent samples in the static condition. Effects of the polymer thickness, drug loading and hydrogel coating on the drug release rates were evaluated in a static condition. The daily drug release was correlated with the minimum effective concentration of Mitomycin C (MMC) for inhibition of human bladder stroma fibroblasts proliferation *in-vitro*. A cellular transwell migration experiment was also carried out to evaluate the feasibility of the coated stents *in-vitro*.

5.2 Experimental Methods

The effect of the MMC concentrations on human bladder stroma fibroblasts (HBdSF) was studied using the procedures in Section 3.4.1. The transwell migration experiment conducted to test the feasibility of the stents *in-vitro*, was described in Section 3.4.2. The drug release rates were evaluated under different conditions using the HPLC method described in Section 3.5.1.

5.3 Assessment of the Drug-eluting Stent in Inhibiting HBdSF Proliferation

The cellular work comprises of two parts. The first part investigated the lowest amount of MMC that was required to induce an inhibitory response to the proliferation of the human bladder stroma fibroblasts (HBdSF) *in-vitro*. The second part was a feasibility study to evaluate if the drugs released from the device was able to inhibit the cells and whether the materials used, namely polyurethane, PLC and PEGDA, as well as the photo-initiator 0.1% (w/v) are cytocompatible with the cells *in-vitro*.

5.3.1 Cell Proliferation Assay to Find the Minimum Effective Concentration and Growth Inhibitory Concentration of MMC to Inhibit HBdSF Proliferations

Using the PrestoBlue® cell viability reagent assay, which is based on resazurin reduction, it was found that the minimum effective concentration of MMC required to inhibit the growth of HBdSF was as low as 0.01 $\mu\text{g/mL}$ (* $p < 0.05$) (Fig 5.1). Moreover, the amount of drugs required to cause 50% reduction in the proliferation of the fibroblast cells, GI_{50} , was 0.115 $\mu\text{g/mL}$ (Fig 5.2). GI_{50} is typically used for measuring the concentration of drugs required to halve the growth of the target cells, hence by knowing the value, it provides information about the efficacy of the drugs against the cells.

No known studies were available with regards to the effect of MMC on human bladder stroma fibroblasts, however there have been studies which provided encouraging basis for the use of low dose MMC in the treatment of ureteric injuries. For instance, Kuroda et al reported that a minimal amount of 0.1 $\mu\text{g/mL}$ MMC was effective to inhibit HeLa cells over 24 hours incubation period *in-vitro*,¹ and Ayyildiz et al reported the success of low dose MMC in the treatment of experimentally induced urethral injury in rat models.²

Fig 5.1 Effect of MMC concentrations on relative HBdSF cell count

Fig 5.2 Amount of inhibition (%) vs Log [MMC]

5.3.2 Transwell Migration Assay to Evaluate the Feasibility of Drug-eluting Stent to Inhibit HBdSF Proliferations

Stents coated with MMC and PEGDA were shown to be effective to inhibit the proliferation of HBdSF (* $p < 0.05$) over the 3 days period (Fig 5.3). This indicates that MMC remained bioactive in inhibiting the fibroblast cell lines as the MMC eluted from the device was not degraded by the plasma treatment and ultraviolet irradiation. Also, the stent without MMC did not yield any significant reduction of HBdSF (** $p > 0.05$). This indicated that the polyurethane, PLC and PEGDA materials were bioinert and cyto-compatible with the cells. Furthermore, 0.1% (w/v) photo-initiator Irgacure was not toxic to the cells. Hence, these results provided a preliminary demonstration for the safety of the device. Nevertheless, tests will be needed to be performed *in-vivo* to assess its safety.

Fig 5.3 Effect of drug-eluting stent samples on the inhibition of HBdSF in transwell migration study

5.4 Tuning and Characterizing Drug Release

The effects of drug release were investigated under various conditions, namely polymer thickness, drug loading and the presence of hydrogel. The daily dosage was estimated and compared with the amount of drugs required to achieve inhibitory effects of the cell *in-vitro*. Finally, the drug release mechanism was elucidated.

5.4.1 Evaluation of the Stability of MMC

The degradation of MMC in the static condition at 0.05 mg/mL in PBS (pH=7.4) commenced on the first day (3% loss). By Day 7, there was approximately 15% loss of drug at 37°C and by the end of the 4th week, close to 50% of the drug still remained stable (Fig 5.4). Georgopoulos and co-workers reported no difference in the long term stability of MMC concentration from a range of 0.02 to 0.4 mg/mL, hence the result would not have been significantly different when a different drug concentration was to

be used.³ For the release study, since the media was replaced on a weekly basis, the 15% loss by Day 7 would be taken into account, instead of the 50% loss by Day 28.

Nevertheless, it is expected that the degradation of the drug could be slowed down since it is encapsulated within PLC polymer matrix and hence the drug protected from the body's harsh physiological environment; Other drug carriers such as poly(Lactide-co-glycolide) (PLGA) or liposomes are used exactly for the similar purpose of keeping the drug intact within themselves, shielded from the enzymes and immune systems in the body.⁴

Moreover, MMC is less stable at $\text{pH} < 6$, however, the study did not investigate the effect of pH on drug stability, because the pH of the ureteric tract and urine can easily be alkalinized by providing the patients with bicarbonate or citrate salts.⁵ Therefore, effort can be made to prolong the effect of MMC during the stent indwelling duration of 4 - 6 weeks.

Fig 5.4 Stability of MMC under 37°C at 0.05 mg/mL in PBS (pH = 7.4)

5.4.2 Effect of Polymer Thickness on Drug Release

In general, the release of the drug was sustained over the 4 weeks period with residual drugs left after the study (i.e. around 45% of MMC cumulatively released by Day 28

for 50 μm thick PLC, shown in Fig 5.5). The deviation from complete release could be due to the lack of time provided for more drug to release and also the degradation of drug in PBS at 37°C (15% per week). This means that it is likely that the drug release can be prolonged even beyond the 4 weeks study.

Slower drug release (%) was observed with greater polymer thickness, in which the release was fastest for 50 μm and lowest for 240 μm (Fig 5.5). This is because for a polymer matrix system, the drug release is diffusion-controlled, hence the release rate is affected by the diffusion path length of the drug carrier, where the thicker the coating, the longer the path length and hence the slower drug is released.⁶ In mathematical terms, the drug release is governed by the Fick's First law of diffusion, in which J (flux) = D (diffusivity of drug) $\times$ dc/dx (where c is the concentration gradient of drug across the diffusion path with the thickness, dx).⁷ Using the Higuchi diffusional model: $M_t/M_o = 2/L (2DC_s t/C_o)^{1/2}$ [rearranged to be $M_t/M_o = 2(2DC_s)^{1/2}/LC_o^{1/2}) t^{1/2}$ in the form of $y = mx + c$, where $y = M_t/M_o$ and $x = t^{1/2}$], when L (a component in the gradient, m) increases, M_t/M_o (drug release amount) and gradient (drug release rate) decreases. Hence, as the polymer thickness increases, path length for the drug diffusion is increased, resulting in reduced drug release rate. Moreover, since no degradation of PLC occurred within the first month, the release is unaffected by degradation.⁸ Nevertheless, with greater polymer thickness, more drug amount (μg) was released as more drugs were loaded due to longer period of spray-coating duration achieved with greater loop numbers. To add on, there was only a very slight increase in calculated drug released (%) when the correction (mentioned in Section 4.3.3) was made. Hence, subsequent analysis and comparisons of drug release under varying conditions in the different studies were made using the uncorrected drug release (%) since the corrections were only an estimation.

Fig 5.5 Effect of polymer thickness on drug released (%)

Fig 5.6 Effect of polymer thickness on drug released (μg)

5.4.3 Effect of Drug Loading on Drug Release

The release rate followed in the order: 2.5% > 5% > 7.5%, where the higher drug loading led to slower % drug release (Fig 5.7). This is a common phenomenon observed by other groups where in the case when the amount of drug loaded into the

matrix (C_0) $\gg$ the saturated amount of drug in the matrix (C_s), it is postulated that more time was required for the larger amount of undissolved or dispersed drugs in the polymer matrix to be dissolved by the PBS solution before they can be released into the surrounding media.⁹ Similar in 5.4.2, applying the Higuchi diffusional model: $M_t/M_0 = 2/L (2DC_s t/C_0)^{1/2}$, where $M_t/M_0 = 2(2DC_s)^{1/2}/LC_0^{1/2} t^{1/2}$, under the circumstance that C_0 (a component in the gradient, m) $\gg C_s$, when the initial drug concentration (C_0) increases, drug release amount (M_t/M_0) decreases and drug release rate (gradient of M_t/M_0 vs $t^{1/2}$ graph) decreases.

However, it is important to note that the net drug released in quantitative term is still greater for the higher drug loading (Fig 5.8), hence from an application point of view for the treatment of stricture, a higher drug loading may still warrant greater efficacy in inhibiting the proliferation of the fibroblasts since the most challenging problem lies with overcoming the highly drug-impermeable urothelium lining.

Fig 5.7 Effect of drug loading on drug released (%)

Fig 5.8 Effect of drug loading on drug released (µg)

Table 5.1 illustrates that C_o is indeed much more than C_s across all concentrations of drugs loaded from 2.5 – 7.5%. The respective initial drug concentration calculated for 2.5%, 5.0% and 7.5% MMC were 0.0256 mg/mg, 0.0526 mg/mg and 0.0811 mg/mg and these values were all much greater than 0.00196 mg/mg (Table 5.1).

To calculate C_o and C_s , the following method is used: The solubility of MMC in PLC polymer was previously experimentally determined by Anthony *et al* to be 2.55 mg/cm³.¹⁰ Using density of PLC = 1300 mg/cm³, the solubility of MMC in 1 mg of PLC is calculated to be 0.00196 mg/mg. The calculation of initial drug concentration (mg/mg) is obtained using the equation:

$$\text{Initial Drug Concentration (mg/mg)} = \frac{\text{Mass of MMC (mg)}}{\text{Mass of PLC (mg)}} \quad (1)$$

Dosage	MMC(mg)/PLC(mg)
C_o (2.5%)	0.0256 mg/mg
C_o (5%)	0.0525 mg/mg
C_o (7.5%)	0.0768 mg/mg
C_s	2.55 mg/cm ³ 0.00196 mg/mg

Table 5.1 Comparison of C_o and C_s for different drug dosages

5.4.4 Evaluation of Daily Drug Dosage

The daily dosage was calculated by dividing the amount of drugs released over the 1 week by the number of days (ie. $n=7$). For instance, if the sample (PBS containing the amount of drugs eluted) was collected for analysis after n number of days, the value derived from the HPLC result in concentration ($\mu\text{g/ml}$) was multiplied with the volume of PBS (5 mL). This value is divided by the number of days n . Hence, over 28 days, 5 time points were collected: After 1 day (1st time point), after 6 days (2nd time point), after 7 days (3rd time point), after 7 days (4th time points) and last 7 days (5th time point). The amounts were all well above 0.01 $\mu\text{g/mL}$, the minimum effective concentration of MMC to inhibit human bladder stroma fibroblasts, as found in the cell assay study (Fig 5.9 - 5.10). Therefore, the design of the coatings in terms of the coating thicknesses and drug loadings were reasonably sufficient. However, this drug amount in the static condition could not fully simulate the condition in a dynamic urine flow system. Thus, the amount of drug release will be elucidated in an *in-vitro* dynamic fluid flow model to be discussed. Furthermore, a transport study was to be carried out to investigate the amount of drugs delivered to the target tissues in an *ex-vivo* model.

Fig 5.9 Amount of drug released daily for different polymer thickness

Fig 5.10 Amount of drug released daily for different drug loading

5.4.5 Modeling and Understanding the Kinetics and Mechanism of Drug Release

Perfect sink condition has been fulfilled for the studies as the released amount (in $\mu\text{g/mL}$) are all >3 times lower than the saturation solubility of MMC in water (0.5 mg/mL).^{11,12} No burst release was observed since release was $<10\%$ in the first day. This amount would correspond to a degree of burst at <1 , where the degree of burst is defined as the ratio of initial release rate to the sustained release rate after which the initial faster release has reduced in rate and rate gradually attained steady state.¹³ A release would be considered as a burst effect if the degree of burst was >1 . The degree of burst was calculated and presented (Table 5.2).

Figure	Parameters that generate the largest release rate for the same figure	Initial release (%)	Sustained release (%)	Degree of burst = Initial/Sustained
5.5	<ul style="list-style-type: none"> • 50 μm polymer thickness • 5 wt% MMC • No PEGDA hydrogel 	6	$40 - 6 = 34$	$6/34 = 0.18$
5.7	<ul style="list-style-type: none"> • 50 μm polymer thickness • 2.5 wt% MMC • No PEGDA hydrogel 	10	$55 - 10 = 45$	$10/45 = 0.22$
5.14	<ul style="list-style-type: none"> • 50 μm polymer thickness • 5 wt% MMC • With PEGDA hydrogel 	10	$40 - 10 = 30$	$10/30 = 0.33$

Table 5.2 Computation of the degree of burst

The absence of an extensive initial burst release is likely due to the hydrophobic nature of PLC which led to minimal uptake of water. The maximum amount of drugs produced within the first day when the release rate was fastest, was recorded at around 25 μg . To ascertain if this amount is considered toxic, a preliminary investigation could be carried out *in-vivo* on a porcine model. The accuracy of the *in-vitro* drug release here is limited by the release behavior recorded in a static condition rather than a dynamic fluid flow conditions. Moreover, in reiteration, the amount of drugs that are expected to be transported to the urothelium is much lower than the amount released from the stent device due to the barrier membrane of the tissue which will restrict drug permeability.¹⁴ These, however, would be addressed in Chapters 6 and 7 subsequently by adopting a dynamic fluid flow condition to elucidate drug release and *in-vivo* porcine model to evaluate drug transport and safety, respectively.

Moreover, analysis of the release profiles demonstrated that the release of drugs followed a non-zero release order since the rates of drugs release decreased with time in a non-linear way. Hence, to determine if the profile follows a first-order release kinetics, Figures 5.5 and 5.7 were re-plotted using cumulative drug release (%) against the square root of time, in order to elucidate the exponent (n value) in the Higuchi diffusional model: $M_t/M_0 = 2/L (2DC_s t/C_0)^n$. The results showed that there is a linear relationship (R^2 values > 0.9), where n exponent = 0.5, indicating that the release kinetic follows the first-order Fickian diffusion mechanism¹⁵. Furthermore, Fig 5.11

and 5.12 attest to the point in 5.4.2 and 5.4.3, that as L (thickness) and C_0 (initial drug concentration) increases, the gradients (slope of M_t/M_0 vs $t^{1/2}$ graphs) decreases. Since no degradation of PLC 70/30 occurred within the study period of 4 weeks as mentioned previously, the drug release pattern was diffusion-controlled and unaffected by degradation.

Fig 5.11 Cumulative drug released (%) for different polymer thickness vs square root of time in static condition

Fig 5.12 Cumulative drug released (%) for different drug loading vs square root of time in static condition

Furthermore, a SEM analysis of the surface morphology of the MMC/PLC coatings of different drug loadings post-release studies experiment were performed, shows a smooth topography (Fig 5.13), indicating that no bulk erosion has occurred within the 4 weeks period. This is reasonable since PLC 70/30 was found to only begin experiencing mass loss after 56 days.⁸ Therefore, this reiterates that the release of drugs can only be due to diffusion and not degradation since a stent is commonly implanted for around only 4 weeks.

Fig 5.13 Smooth surface morphology of MMC/PLC coatings post-release studies

5.4.6 Effect of PEGDA Hydrogel on Drug Release

PEGDA hydrogel did not result in any significant difference in the drug release (%) over the 28 days (Fig 5.14). This is likely attributable to the high equilibrium water content of the PEGDA (>90%) and that the drug MMC is molecularly smaller than the mesh size for 10% (w/v) PEGDA, hence the drug movement through the networks was not hindered. However, there is a slightly greater release within the first few days (<7) when PEGDA hydrogel is present (Fig 5.14). This is likely because MMC has already started to diffuse into the hydrogel during the 1 week of device drying when the hydrogel was still filled with water content.

Fig 5.14 Effect of PEGDA hydrogel on drug released (%) in static condition

5.5 Discussions and Conclusions

PU stent, PLC, PEGDA hydrogel and 0.1% (w/v) Irgacure were cyto-compatible with the human bladder stroma fibroblasts and the BSDES prototype was effective in the inhibition of the cells *in-vitro*.

The amount of drug released from the prototype is tunable, by simply controlling the polymer thickness and drug loading. No burst release of drug was observed. Drug released in this static condition is found to be greater than the minimum effective concentration to treat human bladder stroma fibroblasts *in-vitro* at 0.01 $\mu\text{g/mL}$. The drug release kinetic is found to be first order and controlled by diffusion and is not affected by degradation of the polymer. Furthermore, PEGDA hydrogel did not result in a significant difference in drug eluted in the static condition due to the high water content of the hydrogel which would mimic the environmental condition in the static release medium.

In conclusion, the most suitable release profile for MMC to treat ureteric stricture disease is unknown. However, it is well documented that the urothelial barrier lining is highly impermeable to drug entry,¹⁶ therefore the amount of drugs released from the stent device is expected to be much greater than the amount of drugs that can enter the therapeutic zone of interest (urothelium). Moreover, since the ureteric stricture disease is chronic (long-term), therefore, having a sustained drug delivery that can last 28 days or more, could be highly relevant. Furthermore, the current practice of MMC bolus infusion does not always prevent stricture recurrence and it is accepted that any form of sustained release of MMC has the potential to minimize or even eliminate stricture recurrence. Nevertheless, more studies are required to investigate the drug release in a dynamic fluid flow condition and in *in-vivo* model.

References

1. Kuroda, Y.; Furuyama, J., Physiological and Biochemical Studies of Effects of Mitomycin C on Strain HeLa Cells in Cell Culture. *Cancer Research* **1963**, *23* (5), 682-687.
2. Ayyildiz, A.; Nuhoglu, B.; Gulerkaya, B.; Caydere, M.; Ustun, H.; Germiyanoglu, C.; Erol, D., Effect of intraurethral Mitomycin-C on healing and fibrosis in rats with experimentally induced urethral stricture. *International journal of urology : official journal of the Japanese Urological Association* **2004**, *11* (12), 1122-6.
3. Georgopoulos, M.; Vass, C.; Vatanparast, Z.; Wolfsberger, A.; Georgopoulos, A., Activity of dissolved mitomycin C after different methods of long-term storage. *Journal of glaucoma* **2002**, *11* (1), 17-20.
4. (a) Pisal, D. S.; Kosloski, M. P.; Balu-Iyer, S. V., Delivery of therapeutic proteins. *Journal of pharmaceutical sciences* **2010**, *99* (6), 2557-2575; (b) Langner, M.; Kral, T. E., Liposome-based drug delivery systems. *Polish journal of pharmacology* **1999**, *51* (3), 211-22.
5. Trinchieri, A.; Esposito, N.; Castelnovo, C., Dissolution of radiolucent renal stones by oral alkalization with potassium citrate/potassium bicarbonate. *Archivio italiano di urologia, andrologia : organo ufficiale [di] Societa italiana di ecografia urologica e nefrologica* **2009**, *81* (3), 188-91.

6. Agarwal, S.; Murthy, R. S. R., Effect of Different Polymer Concentration on Drug Release Rate and Physicochemical Properties of Mucoadhesive Gastroretentive Tablets. *Indian Journal of Pharmaceutical Sciences* **2015**, 77 (6), 705-714.
7. Jaimini, M.; Kothari, A. H., *SUSTAINED RELEASE MATRIX TYPE DRUG DELIVERY SYSTEM: A REVIEW*. 2012; Vol. 2.
8. Peng, Y.; Ang, M.; Foo, S.; Lee, W. S.; Ma, Z.; Venkatraman, S. S.; Wong, T. T., Biocompatibility and Biodegradation Studies of Subconjunctival Implants in Rabbit Eyes. *PLOS ONE* **2011**, 6 (7), e22507.
9. (a) Huang, J.; Wigent, R. J.; Bentzley, C. M.; Schwartz, J. B., Nifedipine solid dispersion in microparticles of ammonio methacrylate copolymer and ethylcellulose binary blend for controlled drug delivery. *International Journal of Pharmaceutics* **2006**, 319 (1), 44-54; (b) Benita, S.; Barkai, A.; Pathak, Y. V., Effect of drug loading extent on the in vitro release kinetic behaviour of nifedipine from polyacrylate microspheres. *Journal of Controlled Release* **1990**, 12 (3), 213-222.
10. Anthony H.C. Ng.; Studies of biodegradable drug-eluting tracheal stents. Doctoral Thesis submitted to Nanyang Technological University, School of Materials Science and Engineering. 2012.
11. Phillips, D. J.; Pygall, S. R.; Cooper, V. B.; Mann, J. C., Overcoming sink limitations in dissolution testing: a review of traditional methods and the potential utility of biphasic systems. *Journal of Pharmacy and Pharmacology* **2012**, 64 (11), 1549-1559.
12. Mitomycin C Product Information. Sigma-Aldrich. Available from: https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Product_Information_Sheet/2/m4287pis.pdf
13. Christopher, S. B.; Huang, X., The cost of optimal drug delivery: Reducing and preventing the burst effect in matrix systems. *ACS Symposium Series* **2004**, Vol. 879. Chapter 1, pp 267–282.
14. Douglass, L.; Schoenberg, M., The Future of Intravesical Drug Delivery for Non-Muscle Invasive Bladder Cancer. *Bladder Cancer* **2016**, 2 (3), 285-292.
15. (a) Higuchi, T., Rate of release of medicaments from ointment bases containing drugs in suspension. *Journal of pharmaceutical sciences* **1961**, 50 (10), 874-875; (b) Higuchi, T., Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. *Journal of*

pharmaceutical sciences **1963**, 52, 1145-9; (c) Fick, A., V. On liquid diffusion. *Philosophical Magazine* **1855**, 10 (63), 30-39.

16. Zacche, M. M.; Srikrishna, S.; Cardozo, L., Novel targeted bladder drug-delivery systems: a review. *Research and reports in urology* **2015**, 7, 169-78.

Chapter 6

Results: Part III

Characterization of the Drug Release in In-vitro Dynamic Fluid Flow Condition and Evaluation of the Coated Stent to Enhance Transport of Drug into Explanted Porcine Urothelial Tissues Ex-vivo

This chapter discusses the characterization of the drug release from the stent samples and drug transport into explanted tissues in the dynamic fluid flow condition. An in-vitro model was set up to evaluate the amount of drug released over 1 week. Moreover, an ex-vivo model was set up to elucidate the amount of drug transported to the explanted porcine ureteric tissue over 2 days. In both set ups, the effect of PEGDA was assessed. The results demonstrated that there was more drug release when PEGDA hydrogel was present, which contrasted with the observations in the static conditions. Moreover, it validated the hypothesis that PEGDA hydrogel is necessary to co-opt with the urothelial tissues for enhanced localized drug transfer.

6.1 Introduction

This chapter reports on the characterization of the drug release and transport under the dynamic fluid flow condition. The effect of PEGDA hydrogel was evaluated in both the *in-vitro* and *ex-vivo* models.

6.2 Experimental Methods

The effect of PEGDA hydrogel on drug release and transport was investigated in an *in-vitro* model (Section 3.6.1) over 7-days period and *ex-vivo* model over 2-days period (Section 3.6.2), respectively. The *ex-vivo* study was carried out over shorter duration as it was limited by the decomposition of the ureter tissues. DMSO and sodium azide can slow down decomposition rate but they cannot totally remove it. The drugs in the explanted urothelial tissues were extracted using solid phase extraction (SPE) technique described in Section 3.6.3. The analysis of samples in both studies was conducted using LCMS, described in Section 3.6.4.

6.3 Elucidation of the Drug Release in In-Vitro Dynamic Fluid Flow Condition

The drug release was elucidated over 7 days using an *in-vitro* dynamic fluid flow model and characterized using LCMS.

6.3.1 Effect of PEGDA Hydrogel on Drug Release

A significantly greater amount of drugs was released when the PEGDA hydrogel was present ($3.5 \pm 0.7\%$ by Day 7) than when it was not ($1.0 \pm 0.2\%$ by Day 7) in the dynamic condition (Fig 6.1 - 6.2). This was in contrast with the result observed in the static condition, where there was no significant difference in the amount of drug release with or without hydrogel (except for the first few days; <7 days). The greater

amount of drug released in the presence of hydrogel coating under dynamic condition is likely attributed to the hydrogel acting as a medium, such as the release medium in the static condition. Hence, the PEGDA hydrogel is more effective than the water flowing down the glass tubing in providing the drug coated stent with exposure to medium for drug to partition and release. In other words, the water that flowed down the stent surface (drop-wise at 0.5 mL/min) did not reside on the surface long enough to bring about the release of drugs.

Also, more drugs were released in the static condition compared to dynamic condition *in-vitro* regardless if PEGDA hydrogel was present. Likewise, Pimenta et al demonstrated the same trend of results in which the drug release was faster in a static condition compared to dynamic condition in an experiment comparing the release of diclofenac releasing from hydroxyethyl methacrylate-based hydrogel for soft contact lens under the two conditions.¹ Therefore, the outcome of our study suggested that the hydrogel was essential to increase the drug release from the device in the dynamic urinary tract conditions. However, an *in-vivo* study will be needed in future to determine the therapeutic dosage needed for the treatment to be effective. Nevertheless, the release followed a first order kinetic via diffusion in both static and dynamic fluid flow conditions (Fig 6.3). Nevertheless, this study was unable to demonstrate whether the drugs could be delivered into the urothelial tissues, therefore an *ex-vivo* model was later carried out to mimic the performance of the stent in a dynamic fluid flow condition using explanted porcine ureter.

Fig 6.1 Effect of PEGDA hydrogel on drug released (%) in dynamic fluid flow condition

Fig 6.2 Effect of PEGDA hydrogel on drug released (ng) in dynamic fluid flow condition

Fig 6.3 Cumulative drug released (%) vs square root of time in dynamic fluid flow condition

6.4 Elucidation of the Coated Stent to Enhance Transport of Drug into Explanted Porcine Urothelial Tissues Ex-vivo

The drug release was elucidated over 2 days using an *ex-vivo* dynamic fluid flow model. The drugs in the explanted urothelium were extracted using SPE and drug amount characterized using LCMS.

6.4.1 Effect of PEGDA Hydrogel on Drug Transport into Explanted Porcine Ureteral Tissues

A higher amount of drug was delivered to the explanted tissues for the samples coated with PEGDA hydrogel, with 6793 ± 870 ng/g analyzed, compared to 3143 ± 1546 ng/g for samples without hydrogel (* $p < 0.05$) (Fig 6.4). Hence, the result demonstrated that PEGDA hydrogel was able to augment the delivery of drugs into the urothelium, mediated by the contact of the hydrogel with the tissues. This was consistent with other work in which a drug eluting balloon was found to be more

effective in delivering paclitaxel into the urothelium following 12 hours of contact while no drugs was observed when only 1 hour contact was allowed.² The augmented transport efficacy following an increase in drug residence time was also illustrated in other works.³ Furthermore, comparing the drug-eluting balloon with our BSDES, the adoption of the former in the clinical setting for 12 hours is very unlikely possible since the balloon device is not designed with a hollow lumen like the BSDES which is crucial in allowing the urine to flow through the stent lumen, avoiding chances of urine backflow and hydronephrosis.

The hydrogel did not hinder the mobility of the drugs MMC through the network as the drug is small molecular weight at 334 Da, while 10% w/v PEGDA hydrogel on Day 2 was much bigger in its mesh size at around 60 nm compared to the drug size, as reported in Section 4.4.6. Moreover, MMC was hydrophilic hence it is able to diffuse and move well within the hydrogel that has 90% equilibrium water content (Section 4.4.4). Furthermore, with the hydration of urothelial lining by the flow of PBS and close apposition of hydrogel with the urothelium in the *ex-vivo* set-up, the drug permeation is expected to be augmented.⁴

However, 6793 ng/g was only 4.45% of the drug with respect to the amount loaded (150 µg). Moreover, the *ex-vivo* model was performed using explanted urothelial tissues which could not respond to stenting by abolishing its peristaltic movement in order to dilate, unlike in a natural living physiological system.⁵ Hence, an *in-vivo* experiment was proposed to be performed in later studies.

Fig 6.4 Effect of PEGDA hydrogel on the drug amount delivered to porcine ureteric tissues *ex-vivo* in dynamic fluid flow condition

6.5 Discussions and Conclusions

For the *in-vitro* dynamic condition, significantly more drugs were released cumulatively when hydrogel was present, with 1.5% released by Day 2 (with hydrogel) compared to 0.5% released by Day 2 (without hydrogel). Likewise, for *ex-vivo* dynamic condition, the hydrogel facilitated the transfer of a larger amount of drug to the urothelium, with 4.5% transferred by Day 2 but only 2.1% when hydrogel was absent (Table 6.1). This means that the hydrogel is essential in the design, both for the purposes of achieving close apposition with tissues and also for the enhancement of drug release and transport. This is likely because the hydrogel having a high water content, can mediate the drug partitioning from the coating in the dynamic condition as it exposes the polymer surface with more medium, compared to the stents without hydrogel. Thereafter, because of the hydrogel co-apting with the tissues *ex-vivo*, the drug residence time on the urothelium is increased, hence drug delivery is enhanced.

Furthermore, the difference in the drug released in static condition is slightly higher (10%) when hydrogel is present in the first few days (i.e. Day 2 in this case for the purpose of comparison with other test groups). This is because, as mentioned in

Chapter 5), the drug has likely already begun to equilibrate into the hydrogel prior to immersion into the PBS medium (Table 6.1). However, in reiteration, beyond the first week, this difference in drug released is gradually masked as the hydrogel with high water content closely mimicked the condition of static medium and all pre-trapped drugs have since been released.

To add on, when comparing the *in-vitro* release of the BSDES in dynamic condition with that in the static condition, the formers suffers from the release of a severely reduced drug amount, with only 1.5% (dynamic) compared to 10% (static) by Day 2. This may be due to the different conditions which the samples were exposed to. In the static condition, there is a larger volume of PBS medium (5 mL) compared to the dynamic condition. To calculate the amount of water which BSDES is exposed to, the hydrogel volume is calculated, using the following method: Volume of the 1-cm bilayer coated section (when hydrogel has swelled to contact with the non-dilatory 4 mm-diameter glass tubing which mimicks the ureter) - Volume of the 1-cm stent section without considering hydrogel (assuming that the coating thickness of drug coating in micrometers is negligible) = $\pi (0.4 \text{ cm} / 2)^2 (1 \text{ cm}) - \pi (0.167 \text{ cm} / 2)^2 (1 \text{ cm}) = 0.1 \text{ mL}$ (Fig 6.5). Since the water content was previously calculated to be around 90% (Chapter 4), the volume of water in the hydrogel was approximated to be 0.09 mL. Hence, the drugs were exposed to a significantly larger volume in the static condition (almost 50 times greater) than in the dynamic condition. Nevertheless, the actual volume of water in the dynamic condition which the drugs were exposed to over the 2 days would likely be lower than 0.09 mL, as it takes approximately one day for the hydrogel to swell to 4 mm to achieve apposition with the glass tube. Hence, with the larger volume of water, the bigger diffusion gradient would have resulted in the more extensive release of drugs in static condition than in dynamic condition. In other words, the matrix system has gradually become a reservoir membrane system, with the hydrogel acting as a retarding element (in the dynamic model) for the drug release, compared to pure PBS solution (in the static model).

Fig 6.5 Cross-section view of the bilayer coated section

Conditions	Coated/ uncoated with PEGDA hydrogel	Cumulative drug released (by 2 days) (%)	Cumulative drug transported (by 2 days) (%)
Static (in-vitro)	Uncoated	7.5	N/A
	Coated	10	N/A
Dynamic (in-vitro)	Uncoated	0.5	N/A
	Coated	1.5	N/A
Dynamic (ex-vivo)	Uncoated	N/A	2.1
	Coated	N/A	4.5

Table 6.1 Cumulative drug release (%) for samples coated with and without hydrogel under static and dynamic fluid flow conditions

In conclusion, even though there were three varying conditions (static, dynamic *in-vitro* and dynamic *ex-vivo*), all three of them generated results that concurred with the trend demonstrating the importance of the PEGDA hydrogel in releasing drug from device and enhancing the drug delivery to the urothelium. However, the study was limited by the inability of the ureter to dilate like in a physiological condition, hence an *in vivo* study will be carried out.

References

1. Pimenta, A. F.; Valente, A.; Pereira, J. M.; Pereira, J. C.; Filipe, H. P.; Mata, J. L.; Colaco, R.; Saramago, B.; Serro, A. P., Simulation of the hydrodynamic conditions of the eye to better reproduce the drug release from hydrogel contact lenses: experiments and modeling. *Drug delivery and translational research* **2016**, 6 (6), 755-762.
2. Liourdi, D.; Kallidonis, P.; Kyriazis, I.; Tsamandas, A.; Karnabatidis, D.; Kitrou, P.; Spyroulias, G. A.; Kostopoulou, O. N.; Marousis, K.; Kalpaxis, D. L.; Goumenos, D. S.; Liatsikos, E., Evaluation of the distribution of Paclitaxel by immunohistochemistry and nuclear magnetic resonance spectroscopy after the application of a drug-eluting balloon in the porcine ureter. *Journal of endourology* **2015**, 29 (5), 580-9.
3. (a) Tyagi, P.; Wu, P.-C.; Chancellor, M.; Yoshimura, N.; Huang, L., Recent Advances in Intravesical Drug/Gene Delivery. *Molecular pharmaceutics* **2006**, 3 (4), 369-379; (b) Lazzeri, M.; Spinelli, M.; Beneforti, P.; Malaguti, S.; Giardiello, G.; Turini, D., Intravesical infusion of resiniferatoxin by a temporary in situ drug delivery system to treat interstitial cystitis: a pilot study. *European urology* **2004**, 45 (1), 98-102; (c) Miller, L. J.; Chandler, S. W.; Ippoliti, C. M., Treatment of cyclophosphamide-induced hemorrhagic cystitis with prostaglandins. *The Annals of pharmacotherapy* **1994**, 28 (5), 590-4; (d) Jeong, B.; Bae, Y. H.; Kim, S. W., Drug release from biodegradable injectable thermosensitive hydrogel of PEG-PLGA-PEG triblock copolymers. *Journal of controlled release : official journal of the Controlled Release Society* **2000**, 63 (1-2), 155-63; (e) Ronneberger, B.; Kao, W. J.; Anderson, J. M.; Kissel, T., In vivo biocompatibility study of ABA triblock copolymers consisting of poly(L-lactic-co-glycolic acid) A blocks attached to central poly(oxyethylene) B blocks. *Journal of biomedical materials research* **1996**, 30 (1), 31-40; (f) Jeong, B.; Bae, Y. H.; Kim, S. W., In situ gelation of PEG-PLGA-PEG triblock copolymer aqueous solutions and degradation thereof. *Journal of biomedical materials research* **2000**, 50 (2), 171-7; (g) Wang, A. J.; Goldsmith, Z. G.; Neisius, A.; Astroza, G. M.; Oredein-McCoy, O.; Iqbal, M. W.; Simmons, W. N.; Madden, J. F.; Preminger, G. M.; Inman, B. A.; Lipkin, M. E.; Ferrandino, M. N., Third Place: Increasing Dwell Time of Mitomycin C in the Upper Tract with a Reverse Thermosensitive Polymer. *Journal of endourology* **2013**, 27 (3), 288-293.

4. Wong, R. S.; Ashton, M.; Dodou, K., Effect of Crosslinking Agent Concentration on the Properties of Unmedicated Hydrogels. *Pharmaceutics* **2015**, 7 (3), 305-19.
5. Cummings, L. J.; Waters, S. L.; Wattis, J. A. D.; Graham, S. J., The effect of ureteric stents on urine flow: Reflux. *Journal of Mathematical Biology* **2004**, 49 (1), 56-82.

Chapter 7

Results: Part IV

In-vivo Pilot Study

This chapter discusses the results of the pilot in-vivo study carried out on a porcine model to evaluate if the working prototype was feasible to deliver the drugs to the targeted ureteric tissue segment. Furthermore, presence of side effects of the device was assessed by making observations for signs of hydronephrosis and systemic toxicity. This proof-of-concept study successfully demonstrated that the drugs can be delivered into the highly impermeable urothelial tissues through the coaptation of the hydrogel with the tissue in the in-vivo setting. No urine obstruction occurred as the kidneys remained intact without distension. Negligible drug level was also detected in blood and periureteric fats tissues.

7.1 Introduction

The proof-of-concept study was carried out to evaluate the feasibility of the swellable drug-eluting stent in delivering mitomycin C to the targeted ureteric segments. Signs for hydronephrosis and presence of the drug in the blood were also analyzed. This was conducted on one female porcine. The device was left indwelling in the swine for a day before sacrifice.

7.2 Experimental Methods

The coated stent samples were fabricated using the procedures described in Section 3.2.1 and Section 3.2.7. As elaborated in Section 3.7.1, 7.5% (w/w) of MMC was loaded at 5200 μg and 10% (w/v) PEGDA hydrogel coated. The details of the animal preparation, device implantation procedures, animal sacrifice and post-study analysis were elaborated in Sections 3.7.2 – 3.7.5. Fig 7.1 - 7.2 depict the stent before implantation and Fig 7.3 - 7.4 depict the harvested tissue after 1 day. The stent was successfully inserted by the urologist on Day 0 and removed on Day 1 without encountering resistance or experiencing hydrogel delamination.

Fig 7.1 Bilayer coated stent before implantation

Fig 7.2 Bilayer coated stent before implantation (close-up view)

Fig 7.3 Harvested stent in ureter after 1 day implantation

Fig 7.4 Harvested stent in ureter after 1 day implantation (close-up view)

7.3 Evaluation of Hydrogel Co-aptation with Urothelial Tissues

The IVU images depicted that the radio-opaque agent accumulated at the region just before the hydrogel section of the stent (Fig 7.5). This indicated that the flow of the fluid was blocked by the hydrogel which has swelled and fully contacted with the urothelial walls. After a few seconds, it was seen that the contrast has started to flow through the stent hollow lumen and eventually made its way into the bladder (Fig 7.5). This fluid motion mimicked and demonstrated the path of the urine. Besides, evaluation of the kidney calyx indicated that there was no distension or swelling hence the coated stent did not result in the urine flowing backwards into the kidney since the ureteric tract was not obstructed (Fig 7.5). Furthermore, the expanded hydrogel was vaguely visible on the X-ray due to the uptake of OmnipaqueTM for 2 min before Day 0 stent implantation (Section 3.7.2). This is an added benefit to the clinical procedures as it facilitated the urologist in positioning the coated therapeutic segment at the diseased site and also in making sure that the desired contact between the expanded hydrogel and urothelial tissues was achieved. Moreover, when the ureter was

harvested, the hydrogel inside was found to be intact and firmly adhered onto the stent after 1 day implantation, without signs of delamination.

Therefore, the feasibility and safety of the hydrogel swelling action was clearly demonstrated.

Fig 7.5 X-ray image showing the hydrogel has swelled in the ureter, hence causing the accumulation of contrast media at the region just before the gel segment (top); X-ray image showing that the contrast can flow through the stent hollow lumen to reach the bladder, as an important route of passage from kidney to bladder without potentially causing urine backflow due to obstruction in ureter (hydronephrosis) (bottom)

7.4 Evaluation of Drug Uptake in Tissues

The harvested ureteric segments were analyzed for quantities of MMC using LCMS. There was a significantly higher amount of MMC level found in the middle segment of the stented ureter at 384 ng (in 1.8925 g tissues, 203 ng/g), compared to the other segments where either no stent was implanted or no drugs was coated on the stent sections (Table 7.1, Fig 7.6).

However, this amount of MMC delivered was relatively low. 203ng/g accounted for only 0.05% of the drug with respect to the total drug amount loaded on the device (390 µg). This might be attributed to the highly impermeable barrier membrane of the urothelium and the slow release rate of drugs under the urine flow conditions. The barrier membrane, as discussed in Section 2.1.1, greatly limited the drug permeation due to its stratified epithelium, with a layer of "umbrella cells" at the luminal surface. To reiterate, these umbrella cells are covered by rigid plaques, comprising of uroplakins which brings about the impermeability of the ureteric lining. Coupled by the presence of glycosaminoglycans (GAGs) on the urothelium, a very tight barrier is formed.

Comparatively, the amount was also much lower than that found in the *ex-vivo* setting in which around 6793 ng of MMC in 1 g tissues (4.5%) was detected following 2 days of study (Section 6.4.1) Assuming the same amount of drug was delivered each day, 2.25% of the drugs would have been transported *ex-vivo* per day; This would have been 45 times greater than the amount of drugs delivered *in-vivo* after 1 day implantation (0.05%) (Table 7.2).

Fig 7.6 Graph depicting the drug delivered to selected segments

Sections	MMC (ng) [absolute amount]	Total mass of tissue samples analyzed (mg)
Proximal (Control)	0.46	261.6
Middle (Control)	0.37	251.9
Distal (Control)	0.47	249.5
Proximal (Test)	4.08	1595.7
Middle (Test)	384	1892.5
Distal (Test)	4.44	1839.6

Table 7.1 Drug delivered to selected segments

Model	Drug loaded (μg)	Drug transported (ng)	Drug transported divided by drug loaded (%)
Ex-vivo	150	6793	4.5 (2 days) Estimated: 2.25 91 day)
In-vivo	390	203	0.05 (1 day)

Table 7.2 Comparison between drug transported (%) in *ex-vivo* and *in-vivo* models

The crucial limitation of the *ex-vivo* model in simulating the dilatory response of a stented ureter may have contributed to the difference. In the live animal, it could have taken more time for the ureter to fully abolish all peristaltic motion,¹ thereby resulting in some drugs to be washed away by the urine. Moreover, the living urothelium contains active uroplakins that could have impeded the penetration of agents through it.² To add on, some of the drugs might have also been metabolized in the living tissues or urine *in-vivo* via enzymatic action, particularly the amine groups in Mitomycin C.³ The possible difference in the fluid flow rate and humidity environment between the living porcine ureter and simulated *ex-vivo* model might also have resulted in the difference in drug level detected *in-vivo*.

Nevertheless, an efficacy study has to be conducted to elucidate if the drug amount delivered is sufficient to inhibit any fibroblasts *in-vivo*. To do so, an appropriate method has to be used to create the injury model on the ureter.

In all, the feasibility of the device in delivering drugs locally to the targeted tissue segment was successfully demonstrated, however, the delivery system could be improved to increase the amount of drugs released and delivered.

7.5 Evaluation of Systemic Toxicity

Analysis of the porcine blood showed negligible amount of drugs after a day of implantation. With 0.1 ng/mL of drug measured (Fig 7.7), it might be attributed to the limitation of the technical capability of the LCMS equipment as it typically measures drug level accurately down to 1 ng/mL. Nevertheless, the level detected was exceptionally low, compared to the toxic amount which has been reported to be as high as 400 ng/mL in porcine model.⁴

Fig 7.7 Drug (MMC) level in porcine blood before and after study

7.6 Discussions and Conclusions

This proof-of-concept *in-vivo* study has successfully demonstrated that the bilayer swellable drug-eluting stent (BSDES) is feasible to expand to contact with urothelium in porcine ureter and locally deliver drugs to the targeted ureteric sections. However, the amount of drugs delivered was reduced greatly in the *in-vivo* setting compared to the *ex-vivo* setting (with estimated 2.25% per day *ex-vivo* and 0.05% per day *in-vivo*). This is likely due to the different drug degradation rate in the two conditions, urothelial dilation that occurred *in-vivo* but not *ex-vivo* and the higher urothelial impermeability due to the active uroplakins and more stratified urothelium with tight junctions *in-vivo*. Moreover, safety of the device has been demonstrated as no hydronephrosis (while urine flowed through stent hollow lumen and collected in bladder) and systemic toxicity were observed.

To reiterate, since this is not a device-controlled system, controlled release with the same daily dosage (zero-order) kinetic is not critical. Nevertheless, an efficacy study is required to ascertain if the amount of drugs delivered is sufficient to inhibit the proliferation of fibroblasts. Moreover, a long term feasibility study is required to study the amount of drugs transported at various time points. This is crucial since stricture is a chronic condition, therefore it is important that the device can produce sufficient therapeutic effect over sustained period of time.

References

1. Cummings, L. J.; Waters, S. L.; Wattis, J. A. D.; Graham, S. J., The effect of ureteric stents on urine flow: Reflux. *Journal of Mathematical Biology* **2004**, 49 (1), 56-82.
2. Tyagi, P.; Wu, P.-C.; Chancellor, M.; Yoshimura, N.; Huang, L., Recent Advances in Intravesical Drug/Gene Delivery. *Molecular pharmaceuticals* **2006**, 3 (4), 369-379.
3. Bradner, W. T., Mitomycin C: a clinical update. *Cancer Treat Rev* **2001**, 27 (1), 35-50.
4. (a) Di Stasi, S. M.; Vespasiani, G.; Giannantoni, A.; Massoud, R.; Dolci, S.; Micali, F., Electromotive delivery of mitomycin C into human bladder wall. *Cancer research* **1997**, 57 (5), 875-80; (b) Di Stasi, S. M.; Giannantoni, A.; Massoud, R.; Dolci, S.; Navarra, P.; Vespasiani, G.; Stephen, R. L., Electromotive versus passive diffusion of mitomycin C into human bladder wall: concentration-depth profiles studies. *Cancer research* **1999**, 59 (19), 4912-8.

Chapter 8

Overall Conclusions and Future Work

This chapter discusses the overall results of the research and highlights the importance in the value of the work. Briefly, the BSDES prototype is made successfully, using 10% w/v PEGDA hydrogel and 5 min, 100W plasma treatment. Moreover, the drug release is tunable and can be sustained over 1 month. Also, drugs eluted from the prototype can inhibit human stroma fibroblast from proliferating over 3 days in-vitro. To add on, PEGDA hydrogel successfully augmented the release and transport of drugs under dynamic fluid flow conditions. Lastly, in-vivo study demonstrate the feasibility of the device in porcine mode without side effects observed in the study involving one porcine over one day implantation. Therefore, the results successfully show that BSDES is able to enhance the drug delivered to the drug-impermeable urothelium for the possible treatment or elimination of stricture recurrence. On the other hand, it also raises the potential as a platform technology for other applications and discusses the future work. Finally, it discusses the future work and direction of the project.

8.1 Overview of Research Findings

The hypothesis and objectives illustrated in Section 1.3 shall be recapped here again. Objectives will be evaluated if the goals have been met and whether the hypothesis was hence tested positive.

Hypothesis (recapped):

- A multi-layer swellable coating can be applied to ureteric stents, with no delamination.
- The swollen hydrogel layer would enhance the localized and sustained drug delivery to the urothelium.

Objectives (recapped):

- 1) Develop and fabricate a bilayer swellable drug eluting stent prototype.
- 2) Study the *in vitro* release behavior and drug transfer from BSDES in static, simulated dynamic, and *ex-vivo* dynamic conditions.
- 3) Investigate the feasibility of BSDES in porcine model.

In Chapter 4, it was discussed that the swellable coated stent prototype was fabricated by using spray-coating of the drug/polymer layer and the PEGDA hydrogel layer was later coated onto the first coating layer via photocrosslinking method using a plastic tubing mold with diameter size that matched the ureteric diameter. Moreover, PEDGA hydrogel was successfully synthesized and characterization results demonstrated that 7.5% and 10% hydrogel can maintain mechanical stability over the 4 weeks period without significant mass loss. Their swelling ratio was around 15 - 20 times with respect to their original dry mass and they possessed high equilibrium water content at > 90%. Furthermore, the hydrogel was able to adhere onto the stent polymer coating

over the 4 weeks study by subjecting the hydrophobic polymer coating to surface plasma treatment (100W, 5 min). This treatment time of 5 min was responsible for the significant increase in wettability and surface roughness, which augmented the interfacial adhesion between the hydrophilic hydrogel and hydrophobic PLC stent coating.

In Chapter 5, it was discussed that the drug released from the device prototype was tunable by controlling the polymer thickness and drug loading. Higher polymer thickness led to slower drug release due to longer path length of drug diffusion and greater drug loading resulted in slower drug release due to the greater amount of time needed to dissolve the dispersed drug before they can be released into the environment. This can be understood from using the Higuchi diffusional model $M_t/M_0 = 2/l (2DC_s t/C_0)^{1/2}$, in which larger polymer thickness (l) and larger drug loading (C_0) leads to reduced drug release amount (M_t/M_0) and hence reduced drug release rate (slope of M_t/M_0 vs $t^{1/2}$ graph). Furthermore, drug release was mostly unaffected by the PEGDA hydrogel in the static condition, except for the first few hours as the drugs have started to equilibrate into the hydrogel prior to immersion into the PBS solution. Furthermore, no burst release was observed and the drug release was sustained over the span of 4 weeks period and could likely be prolonged beyond this common stenting period. The sustained drug release characteristic is believed to be a potential deterrent of stricture recurrence. However, the most suitable drug release kinetic to treat ureteric stricture is not known. Nevertheless, the drug release in this study was found to follow the first-order Fickian diffusion-controlled mechanism, without bulk degradation within the study period. Moreover, the eluted drug was able to reduce human bladder stroma fibroblasts over the 3 days study *in-vitro*, while the PEGDA hydrogel and PLC was cyto-compatible with the cells. Also, the concentration of photo-initiator, Irgacure (0.1% w/v) was not toxic to the cells *in-vitro*.

Chapter 6 demonstrated that the PEGDA hydrogel was able to increase the amount of drugs released from device *in-vitro* and transported into the explanted porcine ureteric tissues *ex-vivo* in the dynamic fluid flow condition. This is due to the higher liquid volume the stent drug coating was having contact with when hydrogel was present. Also, PEGDA hydrogel did not hinder the diffusion of the drugs from the PLC

coating to the target since the drugs were smaller in size than the mesh size of the hydrogel networks and the drugs was soluble in the highly hydrated hydrogel with 90% water content. This was in agreement with the reports that accounted that the increase in residence time of the drug due to the close apposition with the targeted tissues can result in an augmentation of drug transfer. This is especially important for urothelium which was known for its low drug penetration due to the tight junctions and uroplakins.

Finally, Chapter 7 depicted that in the 1 day study, the bilayer swellable drug-eluting stent was feasible for use in the *in-vivo* setting, as it can swell in ureter upon absorption of urine and deliver drugs locally to the urothelium upon its apposition with the tissues, without observations of side effects such as hydronephrosis and systemic toxicity.

Therefore, the findings in Chapters 4 - 7 successfully showed that the objectives have been met. Overall, the prototype was made without observation of delamination of hydrogel and the drug transfer was enhanced due to the capability of the hydrogel to achieve apposition with the target tissues. Therefore, the hypothesis of the thesis has been addressed successfully with positive outcome.

8.2 Implications of Research Findings

Firstly, the use of polyethylene glycol diacrylate hydrogels have been shown to be an interesting and useful candidate for its ability to expand in the physiological system to delivery drugs via the co-aptation with tissues. Also, since it is bioinert and not pH responsive, its application is highly relevant for systems such as the urinary tract where patients' urine may differ greatly. Moreover, it is important to note that the hydrogel participates in the expedition of the drug released in the simulated dynamic urinary fluid flow environment while enhancing the permeation of drug transfer through urothelium, one of the highly impermeable membrane. This strengthens the argument for a hydrogel layer over the drug coating for enhanced drug delivery in the ureter. More importantly, the findings were encouraging as

potential solution to overcoming other membrane using the hydrogel, such as in the bladder, colon or intestinal tract for treating cancers or infections. Nevertheless, due to the small mesh size of the hydrogel at less than 100 nm, this platform technology might not be favourable for the delivery of larger molecules such as protein.

Secondly, it is observed that surface roughness has contributed to the improvement of interfacial adhesion. Plasma treatment at longer duration of 5 min helped to achieve the roughness needed for the adhesion between hydrogel and the stent. This is important for a system that cannot rely on chemical methods to achieve adhesion as the drugs on the first coating may be degraded by the use of chemicals.

The treatment of a chronic condition requires a sustained drug delivery system that is able to prolong the delivery of drugs to the diseased site. Mitomycin C encapsulated in poly-L-lactide-co-caprolactone (70/30 PLC) has been demonstrated to display a sustained release over one month or more. This system has been achieved with ease and low cost, using simple method such as spray-coating. The same technique may be applied in other areas where sustained drug delivery is necessary. However, this matrix system may not be suitable in cases when zero order drug release is crucial. Because of the difficulty for the drugs to permeate the urothelium, it is expected that the drug released from device is much greater than drug transported, therefore, the main challenge would be to deliver as much as possible to the target, rather than to invest time in controlling drug release from device.

The research findings have validated the mechanical and functional properties of the device and demonstrated the feasibility of the device in localizing the transfer of drugs into the target tissues. The hydrogel is able to swell in the ureter and contact with the urothelium to transfer drugs locally to the targeted section of the ureter. This is in agreement that the increase in drug residence time on the target can increase the permeation of drugs¹.

To date, there is no existing therapeutic in urology that can deliver drugs to the selected site, hence the ability to achieve this goal is a leap forward in the field for

urologic treatment. Furthermore, the coated stent implanted for a day did not result in any urine backflow into kidney and no systemic toxicity is observed. Moreover, it is clinically easy for the urologists to insert and remove the coated stents. Since the use of the device would not interfere with the current clinical work flow, it is more possible for the favorably impact the different stakeholders, including clinicians, patients, hospitals and insurance companies, by reducing the time for treatment, medical costs and hospitalization stays.

Finally, as currently, there is no available treatments that can enable localized drug delivery in the urology space, hence the success of the device can potentially open new treatment paradigms for other related disorders such as ureteral carcinoma, particularly in the upper tract and renal pelvis. The standard of care for upper urothelial tract cancer involves surgery involving the complete removal of diseased kidney.² The coated stent technology may be a promising renal-sparing minimally invasive treatment.

8.3 Future Studies and Future Research Direction

Though the research findings have demonstrated the feasibility of the device in porcine model for 1 day, more work is required to investigate the long term stability and safety. Future *in vivo* work may involve device implantation for 2 days, 7 days and 14 days to explore drug amount at the time points, using a larger sample size. Moreover, signs of hydronephrosis has to be investigated.

If there are signs of urine backflow into the kidneys, it would be important to consider alternative hydrogel systems that are dissolvable or degradable at faster rate. Other hydrogels could be chitosan, alginate, polyacrylic acid, hyaluronic acid based systems or even thermoreversible hydrogels such as pluronic acid.¹ Chitosan, for instance, was believed to be a good candidate since its positive charges could mediate the interaction with the negatively charged urothelial lining and this mucoadhesion could act as a form of drug permeation enhancer.

To add on, though the drugs are shown to be delivered to the target tissues, the amount is very low at below 1% of the amount loaded. Hence, other alternative drugs may be explored. Paclitaxel, for instance, is more lipophilic and has been shown to be taken up by ureteric tissues more effectively than mitomycin C.³ Moreover, permeability enhancers may also be applied to increase the drug permeation into the tissues.⁴ Drug may also be loaded into carriers such as liposomes for the delivery to the tissues as liposomes were reported to be better than micelles for carrying water insoluble drugs to the bladder.⁵ Besides, liposomes were also thought to have beneficial effect on injured urothelium.¹ This is because the liposomes may modulate the lipid composition of the uroplakins found on the apical surfaces of the umbrella cells in the urothelium.

Furthermore, it is not known if the drug dosage is effective in inhibiting the fibroblasts proliferation in the *in vivo* setting. Different drug concentrations may be coated on the stent and tested to analyze the effects on harvested porcine ureters at different time points. To do so, an appropriate method may be required to induce injury to the ureter at specific section. Histological analysis on the tissues can also be performed to evaluate for signs of tissue necrosis.

Moreover, anti-proliferative drugs such as MMC and paclitaxel are non-selective in inhibiting cell growth hence it is possible that the surrounding urothelial tissues may be affected by the activity of the drugs. To prevent this problem, one solution may be to adopt the use of siRNA. Most importantly, it is important to evaluate if the device may even worsen the recovery of the treated ureter following full thickness incision.

Finally, work can also be performed to evaluate the effects of the coated stent on treating other common indications such as pain and urinary tract infections in which companies such as Boston Scientific has tried to address with their ketorolac and triclosan eluting stents, but to no avail.⁶ Moreover, the coating may also be applied to address kidney stones or even carcinoma in kidney and upper ureteric tract as discussed earlier. To do so, the coatings may be applied onto the upper stent section or the coiled stent section and drugs allowed to be eluted to the diseased site.

Unfortunately, there is no known cancer model in porcine, therefore it is a challenge to investigate its efficacy in the *in vivo* setting. However, the safety and feasibility together with the evidence of the lack of tissue necrotic development in histological analysis, could pave the regulatory approval for tests in first-in-man study.

References

1. Tyagi, P.; Wu, P.-C.; Chancellor, M.; Yoshimura, N.; Huang, L., Recent Advances in Intravesical Drug/Gene Delivery. *Molecular pharmaceuticals* **2006**, *3* (4), 369-379.
2. Grasso, M.; Fishman, A. I.; Cohen, J.; Alexander, B., Ureteroscopic and extirpative treatment of upper urinary tract urothelial carcinoma: a 15-year comprehensive review of 160 consecutive patients. *BJU international* **2012**, *110* (11), 1618-26.
3. Song, D.; Wientjes, M. G.; Au, J. L., Bladder tissue pharmacokinetics of intravesical taxol. *Cancer chemotherapy and pharmacology* **1997**, *40* (4), 285-92.
4. Chen, D.; Song, D.; Wientjes, M. G.; Au, J. L.-S., Effect of Dimethyl Sulfoxide on Bladder Tissue Penetration of Intravesical Paclitaxel. *Clinical Cancer Research* **2003**, *9* (1), 363-369.
5. (a) Connor, R. J.; Engler, H.; Machemer, T.; Philopena, J. M.; Horn, M. T.; Sutjipto, S.; Maneval, D. C.; Youngster, S.; Chan, T. M.; Bausch, J.; McAuliffe, J. P.; Hindsgaul, O.; Nagabhushan, T. L., Identification of polyamides that enhance adenovirus-mediated gene expression in the urothelium. *Gene therapy* **2001**, *8* (1), 41-8; (b) Ratliff, T. L., Identification of pretreatment agents to enhance adenovirus infection of bladder epithelium. *The Journal of urology* **2005**, *173* (6), 2198-9.
6. (a) Krambeck, A. E.; Walsh, R. S.; Denstedt, J. D.; Preminger, G. M.; Li, J.; Evans, J. C.; Lingeman, J. E., A novel drug eluting ureteral stent: a prospective, randomized, multicenter clinical trial to evaluate the safety and effectiveness of a ketorolac loaded ureteral stent. *J Urol* **2010**, *183* (3), 1037-42; (b) Mendez-Probst, C. E.; Goneau, L. W.; MacDonald, K. W.; Nott, L.; Seney, S.; Elwood, C. N.; Lange, D.; Chew, B. H.; Denstedt, J. D.; Cadieux, P. A., The use of triclosan eluting stents effectively reduces ureteral stent symptoms: a prospective randomized trial. *Bju International* **2012**, *110* (5), 749-754.

APPENDIX

1) Raw Data for Contact Angles

(i) No plasma

Samples	Contact angle image
A-1	<div>CA left: 89.3° CA right: 89.3°</div> <p>89.29°</p>
A-2	<div>CA left: 90.0° CA right: 90.6°</div> <p>90.29°</p>
A-3	<div>CA left: 90.6° CA right: 90.2°</div> <p>90.43°</p>

A-4	<div>CA left: 91.1° CA right: 91.4°</div> <div>91.26°</div>
A-5	<div>CA left: 90.9° CA right: 91.2°</div> <div>91.30°</div>

(ii) 10 sec plasma

Samples	Contact angle image
B-1	<div>CA left: 69.9° CA right: 69.9°</div> <div>69.45°</div>

B-2	<div data-bbox="607 191 675 216"><small>CA left: 70.2° CA right: 69.7°</small></div> <p>69.94°</p>
B-3	<div data-bbox="607 611 675 636"><small>CA left: 70.9° CA right: 70.5°</small></div> <p>70.69°</p>
B-4	<div data-bbox="607 1031 675 1056"><small>CA left: 71.7° CA right: 70.8°</small></div> <p>71.25°</p>
B-5	<div data-bbox="607 1451 675 1476"><small>CA left: 71.9° CA right: 71.6°</small></div> <p>71.85°</p>

(iii) 60 sec plasma

Samples	Contact angle image
C-1	<div>CA left: 71.9° CA right: 71.9°</div> <div>71.43°</div>
C-2	<div>CA left: 70.4° CA right: 72.7°</div> <div>71.55°</div>
C-3	<div>CA left: 72.0° CA right: 72.0°</div> <div>71.95°</div>

C-4	<div data-bbox="609 191 675 216"><small>CA left: 71.8° CA right: 72.2°</small></div> <p data-bbox="609 562 688 594">71.98°</p>
C-5	<div data-bbox="609 617 675 642"><small>CA left: 71.7° CA right: 72.5°</small></div> <p data-bbox="609 984 688 1016">72.17°</p>

(iv) 150 sec plasma

Samples	Contact angle image
D-1	<div>CA left: 70.2° CA right: 69.5°</div> <p>70.04°</p>
D-2	<div>CA left: 69.9° CA right: 70.3°</div> <p>70.08°</p>
D-3	<div>CA left: 69.8° CA right: 71.5°</div> <p>70.63°</p>

D-4	<div data-bbox="607 191 672 218"><small>CA left: 70.7° CA right: 71.1°</small></div> <p>70.86°</p>
D-5	<div data-bbox="607 596 672 623"><small>CA left: 70.9° CA right: 71.7°</small></div> <p>71.30°</p>

(v) 300 sec plasma

Samples	Contact angle image
E-1	<div><div>CA left: 67.0° CA right: 66.9°</div> <div>67.30°</div></div>
E-2	<div><div>CA left: 67.7° CA right: 67.3°</div> <div>67.50°</div></div>
E-3	<div><div>CA left: 67.6° CA right: 68.3°</div> <div>67.93°</div></div>

E-4	<div data-bbox="609 191 673 220"><small>CA left: 70.2° CA right: 69.8°</small></div> <p>69.98°</p>
E-5	<div data-bbox="609 611 673 640"><small>CA left: 70.9° CA right: 70.3°</small></div> <p>70.60°</p>

2) Raw data for Shear Adhesion Failure Experiment

(i) No plasma

Samples	Shear Adhesion Failure Experiment
A-1	<p data-bbox="516 810 597 842">0.63 N</p>
A-2	<p data-bbox="516 1272 613 1304">0.679 N</p>
A-3	<p data-bbox="516 1734 613 1766">0.687 N</p>

A-4

0.743 N

A-5

0.904 N

(ii) 10 sec plasma

B-4

1.008 N

B-5

1.046 N

B-6

1.109 N

(iii) 5 min plasma

C-4

1.144 N

C-5

1.113 N

C-6

1.271 N

3) Raw data of AFM

(i) No plasma

Samples	Surface roughness measurement	3D topography												
A-1	<div> <div><p>Image Statistics</p><table><tr><td>Img. rms (Rq)</td><td>3.815 nm</td></tr><tr><td>Img. Ra</td><td>2.904 nm</td></tr><tr><td>Img. Rmax</td><td>39.520 nm</td></tr></table><p>Box Statistics</p><table><tr><td>Rms (Rq)</td><td>3.130 nm</td></tr><tr><td>Mean roughness (Ra)</td><td>2.541 nm</td></tr><tr><td>Max height (Rmax)</td><td>30.591 nm</td></tr></table></div></div> <div>3.815 nm</div>	Img. rms (Rq)	3.815 nm	Img. Ra	2.904 nm	Img. Rmax	39.520 nm	Rms (Rq)	3.130 nm	Mean roughness (Ra)	2.541 nm	Max height (Rmax)	30.591 nm	<div> <div><p>Scanner Headstage Scan size 2.000 μm Scan rate 1.000 Hz Number of samples 256 Image Data Height 100.0 nm Roughness Rms -10783.4 nm Roughness V Pos -42151.1 nm</p><p>view angle light angle</p></div></div>
Img. rms (Rq)	3.815 nm													
Img. Ra	2.904 nm													
Img. Rmax	39.520 nm													
Rms (Rq)	3.130 nm													
Mean roughness (Ra)	2.541 nm													
Max height (Rmax)	30.591 nm													
A-2	<div> <div><p>Image Statistics</p><table><tr><td>Img. rms (Rq)</td><td>5.957 nm</td></tr><tr><td>Img. Ra</td><td>4.544 nm</td></tr><tr><td>Img. Rmax</td><td>55.569 nm</td></tr></table><p>Box Statistics</p><table><tr><td>Rms (Rq)</td><td>5.789 nm</td></tr><tr><td>Mean roughness (Ra)</td><td>4.439 nm</td></tr><tr><td>Max height (Rmax)</td><td>50.630 nm</td></tr></table></div></div> <div>5.957 nm</div>	Img. rms (Rq)	5.957 nm	Img. Ra	4.544 nm	Img. Rmax	55.569 nm	Rms (Rq)	5.789 nm	Mean roughness (Ra)	4.439 nm	Max height (Rmax)	50.630 nm	<div> <div><p>Scanner Headstage Scan size 2.000 μm Scan rate 1.000 Hz Number of samples 256 Image Data Height 100.0 nm Roughness Rms -10783.4 nm Roughness V Pos -42151.1 nm</p><p>view angle light angle</p></div></div>
Img. rms (Rq)	5.957 nm													
Img. Ra	4.544 nm													
Img. Rmax	55.569 nm													
Rms (Rq)	5.789 nm													
Mean roughness (Ra)	4.439 nm													
Max height (Rmax)	50.630 nm													
A-3	<div> <div><p>Image Statistics</p><table><tr><td>Img. rms (Rq)</td><td>7.407 nm</td></tr><tr><td>Img. Ra</td><td>5.128 nm</td></tr><tr><td>Img. Rmax</td><td>36.047 nm</td></tr></table><p>Box Statistics</p><table><tr><td>Rms (Rq)</td><td>7.405 nm</td></tr><tr><td>Mean roughness (Ra)</td><td>5.343 nm</td></tr><tr><td>Max height (Rmax)</td><td>33.645 nm</td></tr></table></div></div> <div>7.047 nm</div>	Img. rms (Rq)	7.407 nm	Img. Ra	5.128 nm	Img. Rmax	36.047 nm	Rms (Rq)	7.405 nm	Mean roughness (Ra)	5.343 nm	Max height (Rmax)	33.645 nm	<div> <div><p>Scanner Headstage Scan size 2.000 μm Scan rate 1.000 Hz Number of samples 256 Image Data Height 100.0 nm Roughness Rms -10783.4 nm Roughness V Pos -42151.1 nm</p><p>view angle light angle</p></div></div>
Img. rms (Rq)	7.407 nm													
Img. Ra	5.128 nm													
Img. Rmax	36.047 nm													
Rms (Rq)	7.405 nm													
Mean roughness (Ra)	5.343 nm													
Max height (Rmax)	33.645 nm													
B-1	<div> <div><p>Image Statistics</p><table><tr><td>Img. rms (Rq)</td><td>2.493 nm</td></tr><tr><td>Img. Ra</td><td>1.791 nm</td></tr><tr><td>Img. Rmax</td><td>22.493 nm</td></tr></table><p>Box Statistics</p><table><tr><td>Rms (Rq)</td><td>2.159 nm</td></tr><tr><td>Mean roughness (Ra)</td><td>1.643 nm</td></tr><tr><td>Max height (Rmax)</td><td>21.877 nm</td></tr></table></div></div> <div>2.493 nm</div>	Img. rms (Rq)	2.493 nm	Img. Ra	1.791 nm	Img. Rmax	22.493 nm	Rms (Rq)	2.159 nm	Mean roughness (Ra)	1.643 nm	Max height (Rmax)	21.877 nm	<div> <div><p>Scanner Headstage Scan size 2.000 μm Scan rate 1.000 Hz Number of samples 256 Image Data Height 100.0 nm Roughness Rms -10783.4 nm Roughness V Pos -42151.1 nm</p><p>view angle light angle</p></div></div>
Img. rms (Rq)	2.493 nm													
Img. Ra	1.791 nm													
Img. Rmax	22.493 nm													
Rms (Rq)	2.159 nm													
Mean roughness (Ra)	1.643 nm													
Max height (Rmax)	21.877 nm													

<div>B-2</div>	<div> <p>Image Statistics</p><table><tr><td>Imp. rms (Rq)</td><td>8.851 nm</td></tr><tr><td>Imp. Ra</td><td>7.238 nm</td></tr><tr><td>Imp. Rmax</td><td>34.905 nm</td></tr></table><p>Box Statistics</p><table><tr><td>Rms (Rq)</td><td>8.777 nm</td></tr><tr><td>Mean roughness (Ra)</td><td>7.222 nm</td></tr><tr><td>Max height (Rmax)</td><td>51.347 nm</td></tr></table></div> <div>8.851 nm</div>	Imp. rms (Rq)	8.851 nm	Imp. Ra	7.238 nm	Imp. Rmax	34.905 nm	Rms (Rq)	8.777 nm	Mean roughness (Ra)	7.222 nm	Max height (Rmax)	51.347 nm	<div> <p>Image Statistics</p><table><tr><td>Imp. rms (Rq)</td><td>8.851 nm</td></tr><tr><td>Imp. Ra</td><td>7.238 nm</td></tr><tr><td>Imp. Rmax</td><td>34.905 nm</td></tr></table><p>Box Statistics</p><table><tr><td>Rms (Rq)</td><td>8.777 nm</td></tr><tr><td>Mean roughness (Ra)</td><td>7.222 nm</td></tr><tr><td>Max height (Rmax)</td><td>51.347 nm</td></tr></table></div>	Imp. rms (Rq)	8.851 nm	Imp. Ra	7.238 nm	Imp. Rmax	34.905 nm	Rms (Rq)	8.777 nm	Mean roughness (Ra)	7.222 nm	Max height (Rmax)	51.347 nm
Imp. rms (Rq)	8.851 nm																									
Imp. Ra	7.238 nm																									
Imp. Rmax	34.905 nm																									
Rms (Rq)	8.777 nm																									
Mean roughness (Ra)	7.222 nm																									
Max height (Rmax)	51.347 nm																									
Imp. rms (Rq)	8.851 nm																									
Imp. Ra	7.238 nm																									
Imp. Rmax	34.905 nm																									
Rms (Rq)	8.777 nm																									
Mean roughness (Ra)	7.222 nm																									
Max height (Rmax)	51.347 nm																									
<div>B-3</div>	<div> <p>Image Statistics</p><table><tr><td>Imp. rms (Rq)</td><td>16.824 nm</td></tr><tr><td>Imp. Ra</td><td>12.483 nm</td></tr><tr><td>Imp. Rmax</td><td>142.42 nm</td></tr></table><p>Box Statistics</p><table><tr><td>Rms (Rq)</td><td>16.079 nm</td></tr><tr><td>Mean roughness (Ra)</td><td>11.989 nm</td></tr><tr><td>Max height (Rmax)</td><td>111.70 nm</td></tr></table></div> <div>16.824 nm</div>	Imp. rms (Rq)	16.824 nm	Imp. Ra	12.483 nm	Imp. Rmax	142.42 nm	Rms (Rq)	16.079 nm	Mean roughness (Ra)	11.989 nm	Max height (Rmax)	111.70 nm	<div> <p>Image Statistics</p><table><tr><td>Imp. rms (Rq)</td><td>16.824 nm</td></tr><tr><td>Imp. Ra</td><td>12.483 nm</td></tr><tr><td>Imp. Rmax</td><td>142.42 nm</td></tr></table><p>Box Statistics</p><table><tr><td>Rms (Rq)</td><td>16.079 nm</td></tr><tr><td>Mean roughness (Ra)</td><td>11.989 nm</td></tr><tr><td>Max height (Rmax)</td><td>111.70 nm</td></tr></table></div>	Imp. rms (Rq)	16.824 nm	Imp. Ra	12.483 nm	Imp. Rmax	142.42 nm	Rms (Rq)	16.079 nm	Mean roughness (Ra)	11.989 nm	Max height (Rmax)	111.70 nm
Imp. rms (Rq)	16.824 nm																									
Imp. Ra	12.483 nm																									
Imp. Rmax	142.42 nm																									
Rms (Rq)	16.079 nm																									
Mean roughness (Ra)	11.989 nm																									
Max height (Rmax)	111.70 nm																									
Imp. rms (Rq)	16.824 nm																									
Imp. Ra	12.483 nm																									
Imp. Rmax	142.42 nm																									
Rms (Rq)	16.079 nm																									
Mean roughness (Ra)	11.989 nm																									
Max height (Rmax)	111.70 nm																									
<div>C-1</div>	<div> <p>Image Statistics</p><table><tr><td>Imp. rms (Rq)</td><td>2.455 nm</td></tr><tr><td>Imp. Ra</td><td>1.598 nm</td></tr><tr><td>Imp. Rmax</td><td>20.029 nm</td></tr></table><p>Box Statistics</p><table><tr><td>Rms (Rq)</td><td>2.507 nm</td></tr><tr><td>Mean roughness (Ra)</td><td>1.738 nm</td></tr><tr><td>Max height (Rmax)</td><td>20.029 nm</td></tr></table></div> <div>2.455 nm</div>	Imp. rms (Rq)	2.455 nm	Imp. Ra	1.598 nm	Imp. Rmax	20.029 nm	Rms (Rq)	2.507 nm	Mean roughness (Ra)	1.738 nm	Max height (Rmax)	20.029 nm	<div> <p>Image Statistics</p><table><tr><td>Imp. rms (Rq)</td><td>2.455 nm</td></tr><tr><td>Imp. Ra</td><td>1.598 nm</td></tr><tr><td>Imp. Rmax</td><td>20.029 nm</td></tr></table><p>Box Statistics</p><table><tr><td>Rms (Rq)</td><td>2.507 nm</td></tr><tr><td>Mean roughness (Ra)</td><td>1.738 nm</td></tr><tr><td>Max height (Rmax)</td><td>20.029 nm</td></tr></table></div>	Imp. rms (Rq)	2.455 nm	Imp. Ra	1.598 nm	Imp. Rmax	20.029 nm	Rms (Rq)	2.507 nm	Mean roughness (Ra)	1.738 nm	Max height (Rmax)	20.029 nm
Imp. rms (Rq)	2.455 nm																									
Imp. Ra	1.598 nm																									
Imp. Rmax	20.029 nm																									
Rms (Rq)	2.507 nm																									
Mean roughness (Ra)	1.738 nm																									
Max height (Rmax)	20.029 nm																									
Imp. rms (Rq)	2.455 nm																									
Imp. Ra	1.598 nm																									
Imp. Rmax	20.029 nm																									
Rms (Rq)	2.507 nm																									
Mean roughness (Ra)	1.738 nm																									
Max height (Rmax)	20.029 nm																									
<div>C-2</div>	<div><p>Roughness Analysis</p> <p>Image Statistics</p><table><tr><td>Imp. rms (Rq)</td><td>10.250 nm</td></tr><tr><td>Imp. Ra</td><td>7.545 nm</td></tr><tr><td>Imp. Rmax</td><td>81.805 nm</td></tr></table><p>Box Statistics</p><table><tr><td>Rms (Rq)</td><td>10.774 nm</td></tr><tr><td>Mean roughness (Ra)</td><td>8.440 nm</td></tr><tr><td>Max height (Rmax)</td><td>81.410 nm</td></tr></table></div> <div>10.250 nm</div>	Imp. rms (Rq)	10.250 nm	Imp. Ra	7.545 nm	Imp. Rmax	81.805 nm	Rms (Rq)	10.774 nm	Mean roughness (Ra)	8.440 nm	Max height (Rmax)	81.410 nm	<div> <p>Image Statistics</p><table><tr><td>Imp. rms (Rq)</td><td>10.250 nm</td></tr><tr><td>Imp. Ra</td><td>7.545 nm</td></tr><tr><td>Imp. Rmax</td><td>81.805 nm</td></tr></table><p>Box Statistics</p><table><tr><td>Rms (Rq)</td><td>10.774 nm</td></tr><tr><td>Mean roughness (Ra)</td><td>8.440 nm</td></tr><tr><td>Max height (Rmax)</td><td>81.410 nm</td></tr></table></div>	Imp. rms (Rq)	10.250 nm	Imp. Ra	7.545 nm	Imp. Rmax	81.805 nm	Rms (Rq)	10.774 nm	Mean roughness (Ra)	8.440 nm	Max height (Rmax)	81.410 nm
Imp. rms (Rq)	10.250 nm																									
Imp. Ra	7.545 nm																									
Imp. Rmax	81.805 nm																									
Rms (Rq)	10.774 nm																									
Mean roughness (Ra)	8.440 nm																									
Max height (Rmax)	81.410 nm																									
Imp. rms (Rq)	10.250 nm																									
Imp. Ra	7.545 nm																									
Imp. Rmax	81.805 nm																									
Rms (Rq)	10.774 nm																									
Mean roughness (Ra)	8.440 nm																									
Max height (Rmax)	81.410 nm																									
<div>C-3</div>	<div> <p>Image Statistics</p><table><tr><td>Imp. rms (Rq)</td><td>8.595 nm</td></tr><tr><td>Imp. Ra</td><td>6.666 nm</td></tr><tr><td>Imp. Rmax</td><td>72.212 nm</td></tr></table><p>Box Statistics</p><table><tr><td>Rms (Rq)</td><td>8.288 nm</td></tr><tr><td>Mean roughness (Ra)</td><td>6.650 nm</td></tr><tr><td>Max height (Rmax)</td><td>61.291 nm</td></tr></table></div>	Imp. rms (Rq)	8.595 nm	Imp. Ra	6.666 nm	Imp. Rmax	72.212 nm	Rms (Rq)	8.288 nm	Mean roughness (Ra)	6.650 nm	Max height (Rmax)	61.291 nm	<div> <p>Image Statistics</p><table><tr><td>Imp. rms (Rq)</td><td>8.595 nm</td></tr><tr><td>Imp. Ra</td><td>6.666 nm</td></tr><tr><td>Imp. Rmax</td><td>72.212 nm</td></tr></table><p>Box Statistics</p><table><tr><td>Rms (Rq)</td><td>8.288 nm</td></tr><tr><td>Mean roughness (Ra)</td><td>6.650 nm</td></tr><tr><td>Max height (Rmax)</td><td>61.291 nm</td></tr></table></div>	Imp. rms (Rq)	8.595 nm	Imp. Ra	6.666 nm	Imp. Rmax	72.212 nm	Rms (Rq)	8.288 nm	Mean roughness (Ra)	6.650 nm	Max height (Rmax)	61.291 nm
Imp. rms (Rq)	8.595 nm																									
Imp. Ra	6.666 nm																									
Imp. Rmax	72.212 nm																									
Rms (Rq)	8.288 nm																									
Mean roughness (Ra)	6.650 nm																									
Max height (Rmax)	61.291 nm																									
Imp. rms (Rq)	8.595 nm																									
Imp. Ra	6.666 nm																									
Imp. Rmax	72.212 nm																									
Rms (Rq)	8.288 nm																									
Mean roughness (Ra)	6.650 nm																									
Max height (Rmax)	61.291 nm																									

	8.995 nm	
--	----------	--

(ii) 10 sec plasma

Sample	Surface roughness measurement	3D topography
D-1	<p>5.542 nm</p>
D-2	<p>2.729 nm</p>
D-3	<p>11.909 nm</p>
E-1	<p>3.135 nm</p>

	13.910 nm	
--	-----------	--

(iii) 5 min plasma

Samples	Surface roughness measurement	3D topography
G-1	<p>18.032 nm</p>
G-2	<p>13.115 nm</p>
G-3	<p>15.801 nm</p>
H-1	<p>12.351 nm</p>

	10.942 nm	
--	-----------	--

4) Calibration Curves

4i. For HPLC analysis of drug release in static condition *in-vitro*

4ii. For HPLC analysis of drug extracted from stent to evaluate drug loading (%) *in-vitro*

4iii. For LCMS analysis of drug release in dynamic fluid flow condition *in-vitro*

4iv. For LCMS analysis of drug transport in *ex-vivo* study and analysis of drug in porcine ureter tissues in *in-vivo* study

4v. For LCMS analysis of drug in porcine blood in *in-vivo* study

Patent

1. Subramaniam Venkatraman, Yingying Huang, Wei Shan Lim, Tsung Wen Chong. (2016). A Stent Assembly and Method of Preparing the Stent Assembly. PCT (Application). WO 2016148648 A1.

Publication

1. Wei Shan Lim, Kenneth Chen, Tsung Wen Chong, Gordon Minru Xiong, William R. Birch, Jisheng Pan, Bae Hoon Lee, Pei Shan Er, Abhijit Vijay Salvekar, Subbu S. Venkatraman, Yingying Huang. (2017). A bilayer swellable drug-eluting ureteric stent: Localized drug delivery to treat urothelial diseases. (Submitted to Biomaterials and Under Review Process)

Conferences

1. Wei Shan Lim (Presenter), Yingying Huang, Tsung Wen Chong, Kenneth Chen, Subbu S. Venkatraman. A Swellable Drug-Eluting Stent to Target Fibrosis-Induced Ureteral Stricture. 33rd World Congress of Endourology and SWL. (2015). Poster.
2. Wei Shan Lim (Presenter), Yingying Huang, Tsung Wen Chong, Kenneth Chen, Subbu S. Venkatraman. A swellable drug eluting stent to target fibrosis-induced ureteral stricture. 10th World Biomaterials Congress. (2016). Poster.
3. Wei Shan Lim (Presenter), Yingying Huang, Tsung Wen Chong, Gordon Minru Xiong, Kenneth Chen, Subbu S. Venkatraman. A Novel Coating for Drug Delivery Stents used to Treat Ureteric Conditions. TechInnovation. (2016). Poster.

4. Wei Shan Lim (Presenter), Yingying Huang, Tsung Wen Chong, Gordon Minru Xiong, Kenneth Chen, Pei Shan Er, Subbu S. Venkatraman. A Swellable Drug-Eluting Ureteral Stent for the Treatment of Urological Diseases. Society for Biomaterials Annual Meeting and Exposition. (2017). Poster.
5. Gordon Minru Xiong (Presenter), Wei Shan Lim, Yingying Huang, Tsung Wen Chong, Kenneth Chen, Subbu S. Venkatraman. Bilayer coated ureteral stent as drug delivery platform to treat ureteric diseases. TechConnect World Innovation Conference and Expo. (2017). Oral.
6. Kenneth Chen (Presenter), Wei Shan Lim, Gordon Minru Xiong, Yingying Huang, Subbu S. Venkatraman, Tsung Wen Chong. A swellable drug-eluting ureteral stent for the treatment of upper tract urothelial carcinoma - a proof of concept in-vivo porcine study. 35th World Congress of Endourology and SWL. (2017). Oral.