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DEVELOPMENT AND EVALUATION OF AN ANTIMICROBIAL URINARY CATHETER

LEANNE FISHER, BSc (Hons), MSc

Thesis submitted to the University of Nottingham for the degree of Doctor of Philosophy

December 2011
DECLARATION

Except where specific reference has been made to other sources, the work present in this thesis is the original work of the author. It has not been submitted in whole, or in part, for any other degree or professional qualification.

Signed…………………….. Date………………………..

Leanne Fisher
ABSTRACT

Over the past few years the healthcare setting has seen a vast increase in the use of medical devices and whilst this may have improved clinical outcomes for patients their increase in use has given rise to an increase in medical - device associated infections. It has been reported that urinary tract infections (UTIs) account for up to 40% of all healthcare associated infections and about 80% of those are associated with catheter use [1]. Urinary catheters are hollow, flexible, tubular devices designed to drain urine when inserted into a patient’s bladder. They are widely used both on patients requiring short - term urinary catheterisation e.g. during and after some types of surgical procedures or long - term urinary catheterisation e.g. due to urinary incontinence. For patients undergoing long - term indwelling urinary catheterisation (LTC) it is almost inevitable that their catheter will become colonised with bacteria and a biofilm (an accumulation of microorganisms and their extracellular products that form a functional, structured community on a surface) [2] develop which can result in a symptomatic or asymptomatic catheter associated urinary tract infection (CAUTI). Infections associated with biofilms are difficult to treat due to the bacteria within the biofilm being insusceptible to antibiotic treatment.

Often to resolve the infection, removal and replacement of the catheter is required and antibiotic treatment if necessary. Certain patients may require their catheter to be changed frequently, often causing considerable distress and morbidity and giving rise to increased medical costs. Biomaterials used to produce long - term urinary catheters that are able to completely resist bacterial colonisation for significant periods, remain elusive. The development of antimicrobial urinary catheters has, however, shown some success in clinical trials but only in the short - term.
This project proposes to modify a silicone urinary catheter used for LTC by impregnating it with a suitable combination and concentration of antimicrobial agents. The aim of the study is to develop an antimicrobial catheter that will provide protection from bacterial colonisation and subsequent biofilm development by the principle organisms involved in CAUTIs over a prolonged period (12 weeks).

Silicone material was processed using an impregnation method. A variety of agents were assessed using drug screening tests to establish their potential duration of antimicrobial activity and ability to prevent bacterial colonisation. The combination of agents showing the most potential were selected and impregnated into the catheter material. They were: rifampicin, sparfloxacin and triclosan. Further testing involved the development of an in - vitro model designed to test the ability of the antimicrobial catheter to resist colonisation following repeated bacterial challenges. The emergence of bacterial resistance was also monitored during this time. In addition, the total antimicrobial content, drug release profiles and uniformity of drug distribution were elucidated using high performance liquid chromatography (HPLC) and time of flight secondary ion mass spectroscopy (ToF-SIMS) respectively. The effect impregnating antimicrobial agents into the catheter had on its surface properties and the impact on mechanical performance of the catheter shaft and balloons were also examined.

Drug screening tests revealed a combination of rifampicin, sparfloxacin and triclosan had the potential to deliver a long duration of protective activity against principal uropathogens. In - vitro model results demonstrated the antimicrobial catheter was able to prevent colonisation by Escherichia coli and Meticillin Resistant
*Staphylococcus aureus* for >12 weeks, *Klebsiella pneumoniae* and *Proteus mirabilis* for 8 weeks but only 8 days against *Enterococcus faecalis*. *K.pneumoniae* and *P.mirabilis* colonised catheters did, however, show an increase in the sparfloxacin and triclosan minimum inhibitory concentrations (MICs), highlighting that the development of bacterial resistance could be an issue. The catheter was found to contain (w/w) 0.006% rifampicin, 0.16% sparfloxacin and 0.17% triclosan of which 19.8% sparfloxacin and 29.9% triclosan were released by a diffusion process over the first 28 days. Rifampicin release was not detected possibly due to low concentrations. With the drug release trend suggesting a continued steady release of sparfloxacin and triclosan above the MIC and with 80.2% of sparfloxacin and 70.1% of triclosan remaining, this would suggest there should be sufficient drug to provide protection from bacterial colonisation over a 12 week duration. However, why the MICs increased as catheters became colonised with *K.pneumoniae* and *P.mirabilis* could be due to a number of factors.

ToF-SIMS revealed the drugs which could be traced (sparfloxacin and triclosan) were mostly uniformly distributed on the catheter surface, with some drug localization being seen which may have added to the initial burst effect and could be important in the prevention of bacterial colonisation during catheter insertion. Surface analysis techniques also showed the incorporation of antimicrobial agents lead to an increase in the surface hydrophilicity but following exposure to an aqueous environment no difference was seen compared to control catheters. As drugs eluted from the catheter the surface topography marginally deteriorated but the impact of this in terms of bacterial colonisation is not thought to be of a clinical significance. No adverse affect to the mechanical performance of the antimicrobial catheter shaft.
or balloon compared to the conventional silicone Foley urinary catheter was shown, indicating that it would be as mechanically stable as the catheter in clinical use and therefore suitable if applied to clinical practice.

Further work on the drug release concentrations and ratios are needed to help overcome the potential of bacterial resistance. The catheter could have a greater effect on reducing bacterial colonisation and potential for resistance development if drug concentrations were adjusted to release at higher concentrations and equal ratios and more data could be gathered if drug release studies were taken to the end point of 12 weeks rather than 28 days. *In - vitro* model challenges using urine as the perfusion medium and a larger array of microorganisms is required and investigations are also necessary to assess the antimicrobial catheters ability to prevent encrustation, a further complication of LTC.

This preliminary study has shown with further work there is potential that the antimicrobial catheter could have a substantial effect on reducing/delaying colonisation by several of the main organisms involved in CAUTIs over a prolonged course. This in turn would help reduce CAUTI rates, reduce the frequency at which catheters need to be replaced and improve the quality of life for patients on LTC.

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Lastly, to my parents. Mum, thanks for all the love, care and support you bring to me each day and for keeping me eating. Dad, ‘13-amp fuses, parasites, ceilings’, I do believe you have not only saved my bacon on many occasions but also encouraged me to believe I could do it. Thank you. This one is for both of you!
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<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AAS</td>
<td>Atomic absorption spectroscopy</td>
</tr>
<tr>
<td>ACN</td>
<td>Acetonitrile</td>
</tr>
<tr>
<td>AFM</td>
<td>Atomic force microscopy</td>
</tr>
<tr>
<td>Ag⁺</td>
<td>Silver ion</td>
</tr>
<tr>
<td>AHL</td>
<td>N-acyl homoserine lactone</td>
</tr>
<tr>
<td>ASD</td>
<td>Artificial spent peritoneal dialysate</td>
</tr>
<tr>
<td>ASTM</td>
<td>American standard test method</td>
</tr>
<tr>
<td>AU</td>
<td>Gold (nanoparticles)</td>
</tr>
<tr>
<td>BS</td>
<td>British standard</td>
</tr>
<tr>
<td>C</td>
<td>Carbon</td>
</tr>
<tr>
<td>CA</td>
<td>Contact angle</td>
</tr>
<tr>
<td>CAUTI</td>
<td>Catheter associated urinary tract infection</td>
</tr>
<tr>
<td>cfu/mL</td>
<td>Colony forming units per millilitre</td>
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<td>Cl³⁷</td>
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<tr>
<td>CLED</td>
<td>Cystine, lactose, electrolyte, deficient agar</td>
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<td>CoNS</td>
<td>Coagulate negative staphylococci</td>
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<td>CTC</td>
<td>5-cyano-2,3,-ditolyl tretazolium chloride</td>
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<td>CVC</td>
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<td>Deoxyribonucleic acid</td>
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<td>Drop size analyzer</td>
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<tr>
<td>E.faecalis</td>
<td>Enterococcus faecalis</td>
</tr>
<tr>
<td>ENR</td>
<td>Enoyl-acyl carrier protein reductase</td>
</tr>
<tr>
<td>EPS</td>
<td>Exopolysaccharide</td>
</tr>
<tr>
<td>ESBL</td>
<td>Extended spectrum beta lactamases</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>EVD</td>
<td>External ventricular drain</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and drug administration</td>
</tr>
<tr>
<td>F</td>
<td>Fluorine ion</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>Ig</td>
<td>Immunoglobulin</td>
</tr>
<tr>
<td>ISA</td>
<td>Iso-sensitest agar</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td><em>Klebsiella pneumoniae</em></td>
</tr>
<tr>
<td>LAAA</td>
<td>Light - acting antimicrobial agent</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>LTC</td>
<td>Long - term catheterisation</td>
</tr>
<tr>
<td>MB</td>
<td>Methylene blue</td>
</tr>
<tr>
<td>MBC</td>
<td>Minimal bactericidal concentration</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
</tr>
<tr>
<td>MHRA</td>
<td>Medicines and Healthcare Products Regulatory Agency</td>
</tr>
<tr>
<td>MIC</td>
<td>Minimal inhibitory concentration</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
</tr>
<tr>
<td>MRSA</td>
<td>Meticillin resistant <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>MSCRAMMs</td>
<td>Microbial surface components recognising adhesive matrix molecules</td>
</tr>
<tr>
<td>MSW</td>
<td>Mutant selection window</td>
</tr>
<tr>
<td>N</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>Na</td>
<td>Sodium</td>
</tr>
<tr>
<td>NAD</td>
<td>Nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>P.aeruginosa</td>
<td><em>Pseudomonas aeruginosa</em></td>
</tr>
<tr>
<td>PAMPs</td>
<td>Pathogen associated molecular patterns</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PDMS</td>
<td>Poly dimethyl siloxane</td>
</tr>
<tr>
<td>PEG</td>
<td>Poly ethylene glycol</td>
</tr>
<tr>
<td>PEO</td>
<td>Poly ethylene oxide</td>
</tr>
<tr>
<td>PEVA</td>
<td>Poly ethylene-co-vinyl acetate</td>
</tr>
<tr>
<td>PFGE</td>
<td>Pulse field gel electrophoresis</td>
</tr>
<tr>
<td>P.mirabilis</td>
<td><em>Proteus mirabilis</em></td>
</tr>
<tr>
<td>PTFE</td>
<td>Polytetrafluoroethylene</td>
</tr>
<tr>
<td>P.vulgaris</td>
<td><em>Proteus vulgaris</em></td>
</tr>
<tr>
<td>Acronym</td>
<td>Definition</td>
</tr>
<tr>
<td>---------</td>
<td>------------</td>
</tr>
<tr>
<td>QCM</td>
<td>Quartz crystal microbalance</td>
</tr>
<tr>
<td>QNM</td>
<td>Quantitative nanomechanical mapping</td>
</tr>
<tr>
<td>QS</td>
<td>Quorum sensing</td>
</tr>
<tr>
<td>RIP</td>
<td>RNAIII - inhibiting peptide</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>RP-HPLC</td>
<td>Reverse-phase high performance liquid chromatography</td>
</tr>
<tr>
<td>S.aureus</td>
<td><em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>SBA</td>
<td>Sheep blood agar</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>S.epidermidis</td>
<td><em>Staphylococcus epidermidis</em></td>
</tr>
<tr>
<td>Si</td>
<td>Silicone</td>
</tr>
<tr>
<td>Si⁺</td>
<td>Silicone ion</td>
</tr>
<tr>
<td>SiH</td>
<td>Silicone-hydrogen ion</td>
</tr>
<tr>
<td>SiO₂</td>
<td>Silicone dioxide</td>
</tr>
<tr>
<td>SPR</td>
<td>Surface plasmon resonance</td>
</tr>
<tr>
<td>SPTT</td>
<td>Serial plate transfer test</td>
</tr>
<tr>
<td>S.saprophyticus</td>
<td><em>Staphylococcus saprophyticus</em></td>
</tr>
<tr>
<td>STC</td>
<td>Short - term catheterisation</td>
</tr>
<tr>
<td>TBO</td>
<td>Toluidine blue</td>
</tr>
<tr>
<td>tK100</td>
<td>Time taken to kill 100% of attached bacteria</td>
</tr>
<tr>
<td>ToF-SIMS</td>
<td>Time of flight - Secondary ion mass spectroscopy</td>
</tr>
<tr>
<td>TSB</td>
<td>Tryptone soya broth</td>
</tr>
<tr>
<td>UPEC</td>
<td>Uropathogenic <em>Escherichia coli</em></td>
</tr>
<tr>
<td>UTI</td>
<td>Urinary tract infection</td>
</tr>
<tr>
<td>UV-Vis</td>
<td>Ultraviolet-visible (spectrophotometry)</td>
</tr>
<tr>
<td>WBC</td>
<td>White blood cell</td>
</tr>
<tr>
<td>WCA</td>
<td>Water contact angle</td>
</tr>
<tr>
<td>XPS</td>
<td>X-ray photoelectron spectroscopy</td>
</tr>
<tr>
<td>ZI</td>
<td>Zone of inhibition</td>
</tr>
</tbody>
</table>
CHAPTER 1
INTRODUCTION AND AIMS
1.0. INTRODUCTION AND AIMS

1.1. Overview

The use of medical devices has become an essential part of modern day medicine and over the past few years the healthcare setting has seen a vast increase in their use. The diversity of such devices is wide, varying from those of a complex nature like automatic implantable cardio defibrillators to more simple devices such as urinary catheters [3]. Whilst medical devices have improved clinical outcomes for patients their increase in use has given rise to an increase in medical - device associated infections [4]. A large array of biomaterials is used in the production of medical devices and one thing they have in common is their inability to prevent bacteria from colonising their surfaces. This is especially true of urinary catheters. Urinary catheters are hollow tubular devices which upon insertion into a patient’s bladder allow for urine to drain. Urinary tract infections (UTIs) are the most common type of hospital - acquired infection accounting for up to 40% of all cases and 80% of those are associated with catheter use [1]. Patients may require catheterisation for short or more prolonged periods of time and with patients undergoing long - term indwelling urinary catheterisation (LTC) it is almost certain that microbes will colonise the catheter surface leading to the formation of a biofilm and a catheter associated urinary tract infection (CAUTI) develop at some point [5]. Such infections are difficult to resolve and can cause considerable morbidity. There is a great deal of interest in devising strategies to minimise biofilm formation to prevent CAUTIs but biomaterials used to produce urinary catheters that are completely resistant to bacterial colonisation for clinically significant periods currently remain elusive. Clearly on the basis of prevalence and associated morbidity, ways of
reducing bacterial colonisation associated with long-term urinary catheters is an area that continues to warrant investigation if CAUTI rates are to decline.

This chapter will provide an introduction to the issues surrounding CAUTIs amongst patients on LTC. The urinary system, reasons for catheterisation and problems associated with catheterisation will be discussed along with the microorganisms involved in infection and their routes of entry. The process of bacterial attachment to catheter surfaces and progression to biofilm state will be explained and issues regarding treatment of CAUTIs as well as ways and methods of trying to reduce CAUTIs will be discussed and the concept of antimicrobial catheters introduced.

1.2. The Urinary System

1.2.1. Components and Functions

The urinary system is one of the body’s major regulatory systems and consists of two kidneys, two ureters, the bladder and urethra (Figure 1.1). It plays vital roles in the excretion of wastes from the body in the form of urine and in the regulation of the amount of water in the body and in turn salts within the blood [6]. It also functions in acid-base balance to maintain correct ionic composition, pH and osmotic concentrations and has a role in endocrine function releasing various amounts of hormones and altering others [6].

The kidneys contain millions of nephrons that filter soluble components from blood and water. Substances the body requires get selectively reabsorbed back into the blood to maintain proper balance whilst waste products that do not get filtered
become secreted into the urine [6]. The ureters are 25 - 30 cm muscular tubes that arise from the renal pelvis and transport the urine towards the bladder. The bladder, a hollow, muscular, elastic sac is located on the floor of the pelvic cavity and every few seconds small amounts of urine are forced into it, typically at a rate of 0.5 - 1 mL/min [7]. It usually accumulates 300 to 400 mL of urine before emptying but can expand to hold up to 1000 mL, although this varies from person to person [6]. As the bladder fills with urine it becomes distended and a layer of smooth muscle fibres within the bladder wall known as the detrusor muscle signals to the parasympathetic nervous system to contract the detrusor muscle which results in the urge to urinate [6]. For the urine to be expelled from the bladder the internal and external sphincters which are usually contracted to prohibit the urine from releasing prematurely, need to be open. The urethra, a tube of smooth muscle lined with mucosa, is the passageway through which urine is discharged from the body during urination. In a female the urethra is about 4 cm in length whereas in a male it is about 20 cm in length [6].

Figure 1.1: Front view of the urinary system [8].
1.2.2. Urine and its Constituents

Urine is a supersaturated solution which is normally transparent, has a slightly aromatic but not unpleasant odour and is yellow to amber in colour. Urine of a turbid nature may indicate bacterial infection or be due to the crystallisation of salts. Depending upon the level of hydration of the body, diet, illness and drugs a person may be taking, the colour of the urine can also vary from near colourless to red/brown. Odour can be affected by certain foods, bacterial contamination and if left to stand outside of the body can lead to a stale, unpleasant smell. The pH of urine is typically near to neutral or slightly acidic but may fluctuate from 4.5 to 8 and is affected by fluid intake, diet, exercise and temperature [9]. Specific gravity, the ratio of the weight of a unit volume of urine to the same volume of distilled water is typically 1.001 to 1.035 (g·cm\(^{-3}\)) and deviations from this may be associated with urinary disorders [6]. In adults the average amount of urine produced in a day is approximately 1 - 2 L [6]. This obviously varies and depends upon factors such as hydration, health, size, environmental factors and emotional state. Producing too much (>2.5 L/day) or too little (<100 mL/day) urine may require medical attention.

The physical properties of normal urine and its constituents (Table 1.1) varies often quite considerably between individuals and within the same individual over time [10]. The urinary system can encounter a variety of problems which may result in the urine being unable to drain from the body in the normal manner. In such instances a hollow tubular device known as a urinary catheter may need to be inserted to allow for more effective drainage.
Table 1.1: Main constituents of normal urine [6].

<table>
<thead>
<tr>
<th>Substance</th>
<th>Origin</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Diet, metabolism</td>
<td>1500 mL</td>
</tr>
<tr>
<td><strong>Organic Substances</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>Protein deamination</td>
<td>30 g</td>
</tr>
<tr>
<td>Creatinine</td>
<td>Metabolism of creatinine in muscle</td>
<td>1.6 g</td>
</tr>
<tr>
<td>Hippuric acid</td>
<td>Liver detoxification of benzoic acid</td>
<td>0.7 g</td>
</tr>
<tr>
<td>Uric acid</td>
<td>Catabolism of nucleic acid</td>
<td>0.7 g</td>
</tr>
<tr>
<td>Ketone bodies</td>
<td>Lipid metabolism</td>
<td>0.04 g</td>
</tr>
<tr>
<td><strong>Inorganic Substances</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td>Diet</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Chloride</td>
<td>Diet</td>
<td>7 g</td>
</tr>
<tr>
<td>Potassium</td>
<td>Diet</td>
<td>4 g</td>
</tr>
<tr>
<td>Phosphates</td>
<td>Diet, metabolism of phosphate-containing compounds</td>
<td>2.5 g</td>
</tr>
<tr>
<td>Sulfates</td>
<td>Diet, metabolism of sulfate-containing compounds</td>
<td>2.5 g</td>
</tr>
<tr>
<td>Ammonia</td>
<td>Deamination of amino acids</td>
<td>0.7 g</td>
</tr>
<tr>
<td>Calcium</td>
<td>Diet and bone demineralisation</td>
<td>0.3 g</td>
</tr>
<tr>
<td>Magnesium</td>
<td>Diet</td>
<td>0.1 g</td>
</tr>
<tr>
<td><strong>Total Solids</strong></td>
<td></td>
<td>About 50 g</td>
</tr>
<tr>
<td><strong>Total Volume</strong></td>
<td></td>
<td>About 1500 mL</td>
</tr>
</tbody>
</table>
1.3. Urinary Catheterisation

1.3.1. History of Urinary Catheterisation

The word catheter in Greek means to let or to send down [11]. Catheterisation of the urinary tract has been performed from time immemorial and a variety of materials have been used to form the tubes. Amongst the materials used to form the shaft of the catheter have been metals such as tin and bronze, silver and gold by the Greeks in the 3rd century, lead and papyrus by the Egyptians and dried reeds and palm leaves by the Chinese [11, 12]. Advancements in catheter design came in the 11th century when more malleable materials were developed and it was realised that by boring holes into the sides of the catheter shaft this helped to facilitate urinary drainage. Latex catheters became popular in the 18th century but initially were weak and friable at body temperature. The process of vulcanization, however, saw the firmness, flexibility and durability of the rubber improve. By the mid 1800’s the first self retaining urinary catheter consisting of a retention balloon made of rubber or woven fabric was formed [12].

1.3.2. Urinary Catheters Today

Urinary catheters nowadays come in a variety of designs, sizes and materials. The most common type of self retaining catheter in use today is the Foley catheter, developed by the American urologist, Frederick E.B. Foley [12]. It has been commercially available since the mid-1930’s and remains relatively unchanged in design today. A typical Foley catheter is 220 - 380 mm in length and consists of a balloon at the proximal tip near to the drainage eyelet and a two - or three - way
outlet at the other end (Figure 1.2). The outlets are for urine drainage, as a means of inflating the balloon with saline to secure the catheter in place within the bladder, and for bladder irrigation if necessary. Catheter size is measured in French units, where 1 French unit = 0.33 mm diameter. Adult catheters vary in diameter from 12 Fr to 30 Fr (4 to 10 mm) with the standard being 14 Fr (4.6 mm) and in balloon size that have a capacity of 5 mL to 30 mL. In the first few decades of use, an ‘open catheter system’ existed whereby the Foley catheters were attached to tubes which drained the urine into buckets [13]. Nowadays, a drainage bag is attached to the urine outlet forming a ‘closed system’ and is either hung beside the patient’s bed or strapped to the patient’s leg. Urine can either drain freely by gravity into the drainage bag or a catheter valve can be fitted into the end of the catheter which may be switched on or off to allow for the urine to drain.

The most widely used materials for Foley catheters are latex or silicone with the first being manufactured from latex. Latex is inexpensive, has good elastic properties but tends to be more prone to bacterial adherence and have more allergic potential than some other materials [14]. Silicone is soft, non-irritating and clinically stable making it ideal for long-term use in the urinary tract [15]. Silicone catheters are more expensive than latex catheters but are inert putting patients at less risk of allergic reaction. Their surface properties make for easy insertion and lower rates of bacterial adherence and encrustation as reported by some studies possibly relating to their smoother surfaces exhibiting less niches for microbial aggregation than latex catheters [14, 16].
1.3.3. Forms of Urinary Catheterisation

There are two main forms of urinary catheterisation: intermittent and indwelling.

1.3.3.1. Intermittent Catheterisation

Intermittent catheterisation is a form of self-catheterisation that is used in individuals who are unable to empty their bladder properly. It is often a method of choice for patients requiring long-term care but is not always suitable if a patient is mentally or physically unable to carry out catheterisation e.g. spinal cord injuries and dementia patients, although catheterisation can also be carried out by a carer. Intermittent catheters differ from Foley catheters described in section 1.3.2 as they do not have retention balloons to hold the catheter in the bladder. Instead, once the flow of urine has stopped the catheter is immediately removed and disposed of or many can be re-used as required.
1.3.3.2. Indwelling Catheterisation

The most common form of catheterisation is indwelling where the catheter is held in the bladder. This category can further be divided into short and long-term use, although this is somewhat arbitrary. Short-term catheterisation (STC) is classed as a catheter being in place for <14 days [5] and long-term catheterisation (LTC) as a catheter remaining in situ for 30 days or more [1]. Indwelling catheters can be inserted in two ways: - either directly into the bladder through a small incision in the wall of the abdomen known as suprapubic catheterisation, or most commonly through the urethra into the bladder, which is what this study concentrates on.

1.3.4. Reasons for Long-term Urinary Catheterisation

It has been reported that up to 25% of patients admitted to a hospital in the UK will require a urinary catheter at some point [13]. Prolonged catheterisation is common not only in patients in hospital but also amongst residents in nursing home settings as well as people within the community. LTC is utilised in the management of a wide range of conditions both in acute and chronic care settings [1]. Frequently older adults with urinary retention or incontinence problems are catheterised. Other indications for LTC are listed in Table 1.2. Many of these patients can be catheterised for months or years.
Table 1.2: Some indications for long-term urinary catheterisation [1].

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder outlet obstruction not correctable medically or surgically</td>
<td></td>
</tr>
<tr>
<td>Intractable skin breakdown caused or exacerbated by incontinence</td>
<td></td>
</tr>
<tr>
<td>Patients with neurogenic bladder and retention</td>
<td></td>
</tr>
<tr>
<td>Patients who have not responded to specific incontinence treatments</td>
<td></td>
</tr>
<tr>
<td>Palliative care for terminally ill or severely impaired incontinent patients for whom bed and clothing changes are uncomfortable</td>
<td></td>
</tr>
</tbody>
</table>

1.4. Problems Associated with Long-Term Urinary Catheterisation

Below are some of the complications that are associated with LTC. Due to these, catheterisation should only be undertaken when all other interventions have been assessed as being inappropriate [15].

1.4.1. Physiological and Social Aspects

Urinary catheterisation can have significant effects upon a patient both socially and physiologically and these are important factors to acknowledge. Catheterisation for the first time can bring about feelings of shame, stigma, and embarrassment which may lead to depression. The loss of control over bodily function can often serve as a reminder to patients of illness, particularly amongst the elderly. Catheter replacement and drainage bag changes can also cause discomfort and distress to a patient and interfere with their daily lives. On a positive note however, following the initial period of adjustment, users do often accept them as part of their life and recognise how a catheter can be convenient and a positive way of freeing them from urine accidents [17].
1.4.2. Trauma

Patients on LTC may present with lower abdominal pain which may amongst other things be related to trauma to the urethra, bladder or surrounding tissue due to incorrect catheter insertion. This can result in urine bypassing the catheter and may cause urethral strictures [18]. Traction on the catheter either by the patient or inadvertently can also cause trauma to the bladder neck and the prostatic bed which can lead to haematuria [11]. Distension or incorrect positioning of the retention balloon on Foley catheters may lead to pressure necrosis and urethral rupture [19]. Over - or under - inflation can cause the balloon to rupture or the catheter to slip out. Good catheterisation techniques, patient education and compliance can help to minimise complications such as trauma.

1.4.3. Bladder Spasms and Contraction

Bladder spasms can cause urine to leak and reflux of any infected urine into the renal pelvis can increase the risk of pyelonephritis, renal scarring and renal failure [13]. Indwelling catheters can also lead to contraction of the bladder, decreasing its capacity and tone [11]. This can make bladder retraining after catheter removal difficult, but can be helped by intermittent clamping of the catheter several days or weeks prior to catheter removal [11].
1.4.4. Encrustation

Encrustation of urinary catheters poses a significant complication to patients on LTC, with incidence rates reported to be as high as 58% [1]. Encrustation is a result of the ionic components in the urine crystallising out onto the surface of the catheter [20]. *Proteus mirabilis* is the organism most commonly involved in encrustation. It produces the enzyme urease, that catalyses the hydrolysis of urea in the urine to ammonia and carbon dioxide [21]. Ammonia dissolves to form ammonium hydroxide, a strong alkali which has a high dissociation constant whilst carbon dioxide dissolves to form carbonic acid, a weak acid which has a low dissociation constant. This results in the generation of alkaline conditions in the urine. This elevation in pH causes calcium and magnesium salts within the urine to precipitate in the form of struvite and hydroxyapatite. The precipitation of calcium and magnesium salts is pH-dependent. It begins at pH 6.7 - 6.8, peaks at approximately pH 7.5 and declines at higher pHs [1]. The analysis of encrusted urethral catheters has shown them to usually be composed of struvite (magnesium ammonium phosphate) and calcium apatite (a form of calcium phosphate) [20, 22]. If encrustation is left to continue, this crystalline material (Figure 1.3) can block the eye holes of the catheter and the lumen. Failure to change the catheter can lead to incontinence due to leakage or painful distension of the bladder due to retention and reflux of infected urine to the kidneys which can trigger symptomatic pyelonephritis, septicaemia and endotoxic shock [21, 23, 24]. Encrustation is of major concern in clinical practice and is often associated with bacterial colonisation of catheters.
1.4.5. Infection

One of the main complications of LTC is infection in the form of CAUTIs (for more detail refer to section 1.5). Bacteria are highly adaptable organisms and biomaterials imitate the basic substrata for which bacteria have developed colonisation and survival strategies [14]. Bacteria that colonise catheter surfaces can develop into biofilms and cause infection. Catheters produced from silicone-based materials are most commonly used in patients undergoing LTC and as with other materials they provide an abiotic surface upon which invading microorganisms can attach and form dense aggregates of biofilms.

In a non-catheterised patient the urethra normally acts as a barrier in preventing microorganisms from entering the bladder. Should binding of bacteria to the bladder
mucosa occur however, it triggers an inflammatory response that results in an influx of neutrophils and sloughing of epithelial cells with bound bacteria [26]. The high flow rates brought about by micturition, typically 10 - 20 mL/s are often successful at flushing out the bacteria as well [27]. This bacterial shearing effect is due to the release of pressure build-up from within the bladder as the sphincters open. The placement of a catheter however disrupts the body’s normal immune mechanisms and prevents effective clearance of microorganisms. It has also been reported [27] that the catheter and balloon orientation can lead to the formation of a urine sump at the bottom of the bladder and an accumulation of bacteria that can bring about infection.

It has been estimated that more than 60% of healthcare - associated infections are biofilm - related [28, 29]. As stated in section 1.1, UTIs are the most common type of hospital - acquired infection (Table 1.3), accounting for 40% of all cases and of those 80% are catheter - associated [1]. The consequences of CAUTIs can be substantial, both medically in terms of morbidity and mortality and economically. Mortality rates associated with CAUTIs are low compared to that of infection in central venous lines but there are a higher absolute number of infections associated with urinary catheters compared to central venous lines and this results in significant morbidity (Table 1.3). A CAUTI acquired in a hospital setting will lead to an increased stay and subsequently increased costs. Patients who develop CAUTIs in primary care settings have greater contact with their GPs, more outpatient appointments and district nurse visits which represents a significant economic burden to the health care sector. In the UK it is estimated that the management of CAUTIs and associated complications due to urinary incontinence cost between £120 and
£2600 per patient for each quarter of a year [12]. Depending on the seriousness of complications that can arise from LTC, this represents an estimated total cost of £1.4 billion per year [12]. With demographic studies suggesting an increasing population within the UK, with the greatest growth seen in the elderly population and catheterisation more frequent in this group, the use of LTC is likely to increase [12].

Table 1.3: Estimated number of indwelling medical devices inserted in the United States per year with estimated infection rates and attributable mortality [15, 30].

<table>
<thead>
<tr>
<th>Device</th>
<th>Estimated No. of devices placed per year</th>
<th>Infection rate (%)</th>
<th>Attributable mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary catheters</td>
<td>&gt;30,000,000</td>
<td>10 – 30</td>
<td>Low</td>
</tr>
<tr>
<td>Central venous catheters</td>
<td>5,000,000</td>
<td>3 – 8</td>
<td>Moderate</td>
</tr>
<tr>
<td>Fracture fixation devices</td>
<td>2,000,000</td>
<td>5 – 10</td>
<td>Low</td>
</tr>
<tr>
<td>Dental implants</td>
<td>1,000,000</td>
<td>5 – 10</td>
<td>Low</td>
</tr>
<tr>
<td>Joint prosthesis</td>
<td>600,000</td>
<td>1 – 3</td>
<td>Low</td>
</tr>
<tr>
<td>Vascular grafts</td>
<td>450,000</td>
<td>1 – 5</td>
<td>Moderate</td>
</tr>
<tr>
<td>Cardiac pacemakers</td>
<td>300,000</td>
<td>1 – 7</td>
<td>Moderate</td>
</tr>
<tr>
<td>Breast implants</td>
<td>130,000</td>
<td>1 – 2</td>
<td>Low</td>
</tr>
<tr>
<td>Mechanical heart valves</td>
<td>85,000</td>
<td>1 – 3</td>
<td>High</td>
</tr>
<tr>
<td>Penile implants</td>
<td>15,000</td>
<td>1 – 3</td>
<td>Low</td>
</tr>
<tr>
<td>Heart assist devices</td>
<td>700</td>
<td>25 – 50</td>
<td>High</td>
</tr>
</tbody>
</table>

NB: It is almost inevitable that patients with long term indwelling urinary catheters will develop an infection.

Scale for attributable mortality: low = <5%, moderate = 2 – 25%, high = >25%.
The duration of catheterisation plays a vital role in the development of infection. The risk of infection increases by approximately 3 - 6% per day so approximately 50% of those with a urinary catheter in place for 7 to 10 days will develop an infection whilst almost all of those on LTC will develop a CAUTI at some point [5]. The definition of a CAUTI as defined by the Center for Disease Control and Prevention [31] includes symptomatic UTI and asymptomatic bacteremic UTI. A symptomatic CAUTI refers to a patient who has had a urinary catheter in place within a 48 hour period before the onset of UTI and who presents with bacteriuria (bacteria in the urine) with counts of >10³ cfu/mL of a predominant pathogen in a sample collected aseptically, who is suffering from associated UTI symptoms or has a high white blood cell (WBC) count in their urine indicative of infection [31-33]. The patient has to demonstrate clinical symptoms aswell as having laboratory evidence of a UTI with no more than 2 uropathogens being present [31]. Progression to >10⁵ cfu/mL can ensue within 24 to 72 hours [1]. Patients with asymptomatic bacteremic UTI show no signs or symptoms of infection but have a urine culture of >10⁵ cfu/mL with no more than 2 species of uropathogens and a positive blood culture with at least 1 matching uropathogen to the urine culture [31]. A small study of patients who were chronically catheterised found that 98% of 605 consecutive weekly urine specimens contained >10⁵ bacteria/mL and 77% of those contained multiple species [34].
1.4.6. Other Complications

A recent article by Maughan et al (2010) [35] found that 79% of patients on LTC within the community experienced at least one complication over two years. 62% had a CAUTI, 55% reported moderate interference with their daily lives, 33% visited an emergency department, 32% found catheter changes to be uncomfortable and 32% had urinary leakage. Most patients with an indwelling catheter experience some kind of complication. Further potential complications are given in Table 1.4.

Table 1.4: Potential complications of urinary catheterisation [11].

<table>
<thead>
<tr>
<th>Urethral/Penile/Scrotal/Prostatic</th>
<th>Renal/Urethral</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urethritis</td>
<td>Vesicoureteric reflux</td>
</tr>
<tr>
<td>Urethral stricture</td>
<td>Pyelonephritis</td>
</tr>
<tr>
<td>Urethral diverticulum/false passage</td>
<td>Renal/ureteric calculus</td>
</tr>
<tr>
<td>Paraphimosis</td>
<td>Obstructive hydronephrosis</td>
</tr>
<tr>
<td>Funiculitis, epididymitis, epididymooorchitis</td>
<td>Chronic renal failure</td>
</tr>
<tr>
<td>Prostatitis</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bladder</th>
<th>General</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic bacteriuria</td>
<td>Haematuria</td>
</tr>
<tr>
<td>Cystitis</td>
<td>Pain</td>
</tr>
<tr>
<td>Vesical, catheter calculi</td>
<td>Urine leakage around the catheter</td>
</tr>
<tr>
<td>Bladder spasms</td>
<td>Systemic sepsis</td>
</tr>
<tr>
<td>Decrease in bladder capacity, atony</td>
<td>Retained, knotted, fractured catheter</td>
</tr>
<tr>
<td>Bladder cancer (transitional cell carcinoma, squamous cell carcinoma) - rare</td>
<td>- rare</td>
</tr>
<tr>
<td>Extraperitoneal perforation of the bladder - rare</td>
<td>Hypersensitivity, anaphylaxis (latex catheters)</td>
</tr>
</tbody>
</table>


1.5. Catheter Associated Urinary Tract Infections

1.5.1. Causative Organisms

There are a multitude of organisms associated with CAUTIs, both of the Gram positive and Gram negative varieties as well as fungi such as the Candida species. Of the bacterial species the major ones involved are *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterococcus spp* (mostly *E. faecalis* and *E. faecium*), and *Staphylococcus spp*. (mostly *S. aureus* and Coagulase Negative Staphylococci (CoNS)) [1, 27]. In addition, depending on the patient’s health and immune status many opportunistic pathogens may be involved, for example *Providencia stuartii* and *Morganella morganii* [27]. Strains producing extended-spectrum-β-lactamases (ESBL < ampC) and Meticillin Resistant *Staphylococcus aureus* (MRSA) can also be troublesome [36]. In the early stages of infection, a single bacterial species may colonise the catheter. The longer a catheter remains in place, the more likely infection with complex mixed communities of microorganisms will develop [37]. Table 1.5 summarises the bacterial species isolated from a set of 106 urinary catheter biofilms: 14 species were commonly found of which most biofilms contained mixed bacterial communities of more than 2 species [23].
Table 1.5: The number (%) of bacterial species isolated from 106 catheter biofilms [23].

<table>
<thead>
<tr>
<th>Species</th>
<th>All catheter biofilms</th>
<th>Mixed-species biofilms (76 catheters)</th>
<th>Single-species biofilms (30 catheters)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>38 (35.9)</td>
<td>31 (40.8)</td>
<td>7 (23.3)</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>36 (34.0)</td>
<td>34 (44.7)</td>
<td>2 (6.7)</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>33 (33.1)</td>
<td>31 (40.8)</td>
<td>2 (6.7)</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>32 (30.2)</td>
<td>26 (34.2)</td>
<td>6 (20.0)</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>19 (17.9)</td>
<td>18 (23.7)</td>
<td>1 (3.3)</td>
</tr>
<tr>
<td><em>Morganella morganii</em></td>
<td>14 (13.2)</td>
<td>11 (14.5)</td>
<td>3 (10.0)</td>
</tr>
<tr>
<td><em>Providencia stuartii</em></td>
<td>11 (10.4)</td>
<td>9 (11.8)</td>
<td>2 (6.7)</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>11 (10.4)</td>
<td>10 (13.2)</td>
<td>1 (3.3)</td>
</tr>
<tr>
<td><em>Enterobacter cloacae</em></td>
<td>9 (8.5)</td>
<td>7 (9.2)</td>
<td>2 (6.7)</td>
</tr>
<tr>
<td><em>Klebsiella oxytoca</em></td>
<td>9 (8.5)</td>
<td>8 (10.5)</td>
<td>1 (3.3)</td>
</tr>
<tr>
<td><em>Providencia rettgeri</em></td>
<td>5 (4.7)</td>
<td>4 (5.3)</td>
<td>1 (3.3)</td>
</tr>
<tr>
<td>Coagulase-negative staphylococci</td>
<td>5 (4.7)</td>
<td>4 (5.3)</td>
<td>1 (3.3)</td>
</tr>
<tr>
<td><em>Citrobacter species</em></td>
<td>4 (3.8)</td>
<td>4 (5.3)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>3 (2.8)</td>
<td>2 (2.6)</td>
<td>1 (3.3)</td>
</tr>
</tbody>
</table>

1.5.2. Microbial Entry

Microbes can gain access to a urinary catheter via the intraluminal or extraluminal routes. Entry to the catheter lumen is primarily through failure of the closed drainage system. To drain the urine from the collection bag the drainage tube must be routinely opened. Should the drainage tube become contaminated with bacteria, they may gain entry to the drainage bag and either by capillary action or if the bag is raised during movement bacteria can ascend the catheter lumen and travel to the bladder to establish infection [13, 38, 39]. The source of organisms that access via the intraluminal route may originate from the skin of the patient or may be transitory, from a healthcare worker.
Organisms that gain entry to the urinary tract via the extraluminal route are primarily endogenous in nature and originate from the patient’s own gastrointestinal tract [38]. Organisms can be introduced at the time of catheter insertion if inadequate asepsis is used and track along the outside of the catheter. Contaminating organisms from the periurethral area may also ascend along the external surface of the catheter and establish infection [33, 39]. Despite meticulous attention to good hygiene practices, the space between the external surface of the catheter and the urethra gives bacteria the direct chance to enter the bladder via the outside of the catheter [13, 40, 41].

Tambyah et al (1999), [42] examined the possible routes of entry infecting organisms take to access the catheterised urinary tract. They found that 66% of infections were acquired extraluminally and 34% intraluminally. They also reported that Gram positive cocci (enterococci and staphylococci) and yeasts were isolated more from the extraluminal pathway whereas Gram negative bacilli were equally cultured from extraluminal and intraluminal routes. Both the external and internal routes of entry are thought to be of importance.

Sabbuba et al (2003) [24] investigated the epidemiology of catheter - associated P.mirabilis infection using Pulse Field Gel Electrophoresis (PFGE) and found that strains isolated from crystalline biofilms were identical to those in samples taken from the patients urine. Further to this Mathur et al (2005) [43] examined urine and faeces from eighteen patients on LTC whom had P.mirabilis infections and suggested that faecal strains may contaminate the perineum and urethral meatus and ascend the external surface of the catheter to cause bacteriuria, encrustation and biofilm development.
1.5.3. Biofilm Formation

The catheterised urinary tract provides the ideal conditions for microbial adhesion and the development of biofilm populations both on the inner and outer surfaces of the device [16]. The definition of a biofilm is “an accumulation of microorganisms and their extracellular products that form a functional, structured community on a surface” [2]. The development of a biofilm is a series of complex but discrete and well - regulated steps whereby highly complex structures designed to maximise survival and spread are formed [27, 44]. The molecular mechanisms of biofilm formation differ between organisms but the stages of biofilm development are similar [44]. Figure 1.4 depicts the stages of biofilm development and sections 1.5.3.1 - 1.5.3.4 describes the stages involved in more detail.

![Figure 1.4: Biofilm formation and development](image_url)
1.5.3.1. Conditioning Film

Once a catheter or any medical device has been inserted into the body, surfaces become exposed to bodily fluids, such as blood, saliva, mucus, or in the case of urinary catheters, urine. As a result, urinary components adsorb onto the surface of the catheter to form a conditioning film layer [10]. Surface conditioning films are rapidly adsorbed whenever materials are exposed to natural and in-vitro solutions containing organic molecules. The movement of organics from bulk phase to the surface is primarily the result of molecular diffusion. Theoretical and experimental studies have indicated that molecular diffusion occurs rapidly, resulting in significant organic deposits after just 15 minutes [45]. The conditioning film does not cover the whole of the material surface but forms a mesh-like covering [46]. A conditioning film formed on a urinary device is largely composed of proteins e.g. albumin, Tamm-Horsfall Protein and α1-microglobulin, electrolyte components and other unidentified molecules [10]. The creation of it is crucial as many organisms do not have mechanisms that allow them to adhere directly onto bare implant surfaces [47]. Conditioning films can mask the original chemical surface composition and properties of the biomaterial as the matrix of adsorbed products provides receptor sites for bacterial adhesion which can lead to altered microbial adhesion profiles [48]. The consequence of conditioning films for the surface properties of a biomaterial is therefore of considerable significance in the infective process [49]. As stated in section 1.2.2, the composition of urine varies between individuals and within the same individual over time and it has been reported that such differences in the characteristics/composition of urine can alter the receptivity of surfaces to bacteria [10].
1.5.3.2. Bacterial Attachment

After the deposition of a conditioning film, the next step involves the approach and attachment of planktonic organisms to the catheter surface. Planktonic bacteria move to a surface by physical forces such as Brownian motion, van der Waals forces, gravitational forces, hydrophobic interactions and electrostatic charge effects [50]. These physical forces can further be divided into long-range interactions and short-range interactions [50]. Bacteria are first transported to a surface by long-range interactions (>50 nm). As the cells and surface become closer, the short-range interactions (<5 nm) dominate. To understand the forces that determine microbial adhesion, researchers have investigated whether the same physicochemical interactions that determine the deposition of nonliving colloidal particles are the same as those for bacterial attachment to surfaces.

Three theoretical approaches have been used: the Derjaguin, Landau, Verwey, Overbeek (DLVO) theory, the thermodynamic approach and the extended DLVO theory. The DLVO theory describes the net interaction between a bacterial cell and a flat surface as a balance between two additive factors, resulting from van der Waals interactions (attractive) and repulsive interactions from the overlap between the electrical double layer of the cell and surface [51]. The thermodynamic approach takes into account the attractive and repulsive forces but expresses them collectively in terms of free energy [51]. It requires the estimation of a value for thermodynamic parameters i.e. surface free energy of bacterial and substratum surfaces and of the suspending solution [52]. The extended DLVO theory builds on the DLVO theory to include hydrophobic and hydrophilic interactions and osmotic interactions [51].
Only a small number of attached organisms are needed to initiate biofilm formation. The success of this depends on many factors, of bacterial origin and environmental or host derived [27]. Bacterial attachment is regulated through specific interactions between bacterial surface components (adhesins) and host cell or catheter surface receptors. The bacterial adhesins initiate attachment by recognising host cell surface and extracellular matrix components such as proteins, glycoproteins, glycolipids and carbohydrates on the surface of the host cell or on the catheter [53]. Gram negative bacteria produce a variety of adhesins, such as flagellum, fimbriae, pili and nonfilamentous adhesins [53]. Uropathogenic *E.coli* strains (UPEC) for example, use specific adhesins including Type 1 and P fimbriae [53]. *P.mirabilis* produces various fimbriae such as MR/K and haemagglutinins which are involved in the colonisation of the urinary tract [53]. *S.aureus* adheres to the host surfaces mediated by surface adhesins (MSCRAMM’s: microbial surface components recognising adhesive matrix molecules) [54]. Many of the Gram negative uropathogens use flagella and type IV pili motility to facilitate the spread of infection.

Environmental properties of a materials surface such as hydrophobicity, charge and energy as well as properties of the surrounding medium such as flow rate, temperature and osmolarity also play important roles in the ability of bacteria to attach to a biomaterial [27]. In response to bacterial invasion is the hosts innate immune system and natural constituents within urine e.g. urea, salts, antimicrobial peptides, which act to eliminate bacteria. The initial attachment is reversible and to progress to irreversible adhesion, permanent bonds need to form between complementary molecules on a cell/catheter surface. Attached cells upregulate genes that direct the production of an accumulation of proteins and exopolysaccharides.
(EPS). EPS are high molecular weight polymers composed of monosaccharides and some non-carbohydrate substituent’s that are secreted by microorganisms into the surrounding area. It acts to entrap and protect the bacteria by irreversibly fixing cells to one another and to the catheter surface [44, 55].

1.5.3.3. Proliferation/Maturation

The arrival and irreversible attachment of the first bacterial cells facilitates the arrival of other cells as more diverse adhesion sites are provided. As the matrix builds and cells undergo exponential binary division, daughter cells spread to form cell clusters. Progression of bacterial colonisation results in further proliferation and production of EPS which embeds aggregated cells and microcolonies are formed. As the biofilm matures, a 3D structure is formed along the catheter appearing as patchy networks or as a continuous layer [44]. A coherent structure consisting of pillars separated by fluid filled spaces where organisms receive nutrients and wastes diffuse away is formed [27]. Bacteria such as *P.aeruginosa* and *K.pneumoniae* produce copious amounts of EPS and form mucoid biofilms that can occlude catheter lumens [16].

Biofilms evolve according to the biochemical and hydrodynamic conditions and availability of nutrients in the surrounding area [27]. The success of microorganism survival is mostly due to their ability to sense small environmental changes and make rapid adjustments. Cells within a biofilm constantly assess their own population dynamics and adjust their phenotypes accordingly [27]. Once a certain density of bacteria has been reached, a network of signals are produced by the biofilm cells allowing for communication between each other. This type of cell-cell communication is known as quorum sensing (QS) [27]. A variety of molecules can
be used as signals, e.g. oligopeptides in Gram positive organisms, N-Acyl Homoserine Lactone (AHL) in Gram negative organisms and a host of autoinducers. Quorum sensing is capable of altering a number of biological responses including the development of biofilms. For example, furanones found in the marine alga *Delisea pulchra* have antimicrobial and QS inhibitory effects in several Gram negative bacteria. The development of synthetic analogs has been shown to effectively block *P. aeruginosa* biofilm formation and further development of furanone-based drugs may block QS systems in many other Gram negative bacteria [56]. Cirioni *et al* (2007) [57] found that urethral stents coated in the QS inhibitor RNAIII-inhibiting peptide (RIP) that were implanted in rat bladders suppressed *S. aureus* biofilm formation on the stent and in the urine and was particularly effective when combined with teicoplanin. However, Otto (2004) [58] reported that the effects of RIP is due to its physico-chemical properties (detergent like action) rather than by a regulatory process that involves QS, so is actually a coating process opposed to a QS mechanism. Vuong *et al* (2004) [59] reported that inhibition of the QS system in staphylococci has been shown to enhance biofilm development in rabbit models and by disabling the QS system is likely to enhance the success of *S. epidermidis* infection of indwelling medical devices. The use of molecules that interfere with QS shows some promise in counteracting microbial adaptation to host environments [60] but further investigations into specific pathogens and types of infections are required.

1.5.3.4. Detachment and Dispersal

The formation of a biofilm is a universal strategy for microbial survival and to prevent density-mediated starvation within a mature biofilm, cells must detach and diseminate. The detachment and dispersal process acts to spread and prolong
infections making them persistent and greatly increased in severity [27]. Biofilm cells may be dispersed by the shedding of daughter cells from actively growing cells, by detachment due to lack of nutrients and migration to more supportive growth areas or by QS whereby an increase in cell density induces cell – cell signalling to direct the chemical degradation of EPS sending clumps of biofilm into the circulation [61]. Shearing (erosion/shearing, sloughing or abrasion) due to the flow effects urinary catheters are under is also a mechanism whereby cells become dispersed. It has been suggested that detachment is probably species specific and the mode of dispersal may affect the phenotypic characteristic of the organism [61]. Detached biofilm cells can reattach to other parts of the catheter surface and seed the urine and bladder with pathogens which may get into the systemic circulation and cause bloodstream infections [61]. This shedding effect is of most clinical importance as it can result in organisms remaining in the urinary tract even following device removal and replacement.

### 1.6. Microbial Resistance

The microbiological aspects of biofilms associated with symptomatic CAUTIs in terms of the species identified and their susceptibility can be misleading as they may reflect the organisms that were free floating when the urine specimen was collected as opposed to being isolated from a biofilm on a section of catheter [26]. Antibiotic treatment of planktonic bacteria may suppress symptoms of infection but concentrations fail to act on bacteria embedded in the biofilm [62]. That being said, their ability to at least slow biofilm formation and clear planktonic bacteria is of critical importance and their use continues until better therapeutic options become available [14]. In addition, the use of antibiotics prior to device placement or
between device changes is crucial in helping control infection. It is normal for symptomatic patients to have their catheter replaced and by doing this removes the biofilm but detached bacteria may re-seed the urine so it is usual to prescribe a course of antibiotics that are based on the microbiology results of the urine culture [14]. However, when antibiotic treatment is stopped, recurrent infection is common. Of major concern in the formation of biofilms is that the cells embedded in the depths of the biofilm are insusceptible to antibiotics and are inherently protected from host defences. Studies have shown that some organisms within biofilms require >1000 fold higher concentration of antibiotics compared to their planktonic forms in order to kill them [62].

As the understanding of biofilm resistance developed, three main mechanisms were hypothesised:

- **Hypothesis 1:** the slow penetration of antimicrobial agents into the depths of a biofilm is probably a major factor in conferring resistance [62]. The EPS may retard the antibiotic from reaching buried cells within the biofilm and the matrix may also inhibit the transfer of the antibiotic by destruction, chelation or direct blockage [27]. This may result in sub-lethal concentrations of antibiotic and allow for the build-up of resistance mechanisms [27].

- **Hypothesis 2:** the development of altered microenvironments within a biofilm may lead to resistance. The decrease in oxygen within the deeper layers of the biofilm can lead to anaerobic niches and some antibiotics lose activity under these conditions [62]. An accumulation of waste products can lead to an alteration in pH which can antagonise the action of antibiotics and
waste product levels or depletion of substrates can also cause the bacteria to enter a non-growing state [62]. Several groups of antibiotics are effective only on rapidly growing bacteria. A change in the osmotic environment within a biofilm may also lead to resistance by changing the proportion of porins in a way that reduces cell envelope permeability to antibiotics [62].

- **Hypothesis 3:** the production of a sub-population of microorganisms forms a highly protected dormant state of cells that are similar to bacterial spores. This is supported by studies that found resistance in newly formed biofilms where the bacteria possess no particular resistance mechanisms to survive. These bacteria remain resistant upon repeat antimicrobial treatment and resume growth once the concentration of antibiotic drops to a sub-inhibitory concentration. This may be a further mechanism that may explain why these persister type cells have reduced susceptibility to antibiotics [27].

Proctor *et al* (1995) [63] report on small-colony variants (SCVs) of *Staphylococcus aureus* being cultured from patients with persistent and relapsing infections. SCVs have been associated with some foreign body-associated infections, in particular with chronic catheter-related infections. SCVs are a slow growing sub-population of bacteria which are better able to persist in mammalian cells and are less susceptible to antibiotics than their wild type counterparts [64]. They reflect impaired respiratory metabolism due to disruptions in the synthesis of constituents associated with the electron transport system and have unusual biochemical profiles [63, 64]. Clinically SCVs can be difficult to detect due to their atypical morphology and slow growing nature, but if detected, this may also be a reason for resistance to antibiotic treatment in clinical practice.
1.6.1. Host Immune System Evasion

In addition to cells within a biofilm being resistant to antimicrobial agents they are also inherently protected from host defence systems. To maintain infection in the urinary tract organisms must evade the host immune response [53]. The urinary tract, however, contains receptors that recognize intruding pathogens by their invariant pathogen associated molecular patterns (PAMPs) and of the immune surveillance molecules, toll-like receptors are able to detect PAMPs [65]. These act to mobilize appropriate immune defence systems which help to eliminate bacteria from the urinary system. Uropathogenic bacteria, however, excrete a variety of virulence factors that enable them to inhibit certain host functions and promote colonisation. In Gram negative bacteria, the production of capsules plays a role in immune evasion as they resist phagocytosis. During the course of a UTI, immunoglobulin (Ig) antibodies secreted by the host in response to infection recognise antigenic components of uropathogens. However, some bacteria break down host Ig using Ig proteases and other host defence systems like complement (Clq and C3) [53]. Other virulence factors associated with Gram negative bacteria include toxins such as haemolysin and lipopolysaccharide (LPS). LPS acts as an endotoxin and elicits a strong immune response and is in part responsible for the clinical manifestations during infection brought about through cytokine release. Figure 1.5 [53] depicts the virulence factors of the Gram negative uropathogens *E.coli* and *P.mirabilis*. This evasion of the host immune system in response to infection by the biofilm cells along with their insusceptibility to antimicrobials makes treatment difficult and costly.
1.6.2. Symptoms

Although long-term urinary catheterisation is synonymous with bacteriuria, bacteriuria is not synonymous with symptomatic UTI [26]. Over 90% of cases are asymptomatic [32]. Current evidence suggests that asymptomatic patients should not be treated unless the patient is immunosuppressed, at risk of bacterial endocarditis, pregnant or due to undergo urinary tract instrumentation, as these infections clear up once the catheter is removed [53, 66].
The symptoms a patient with symptomatic CAUTI may experience can vary from mild to severe. They may feel pressure in their lower pelvis, have urine that has an odour to it or is cloudy which sometimes may contain blood (haematuria), and a patient may also experience leakage of urine [67]. Additional symptoms may include:- fatigue, fever, vomiting, mental changes or confusion [67]. Often in the elderly, mental changes or confusion are the only symptoms of a possible CAUTI [26]. Symptoms however may be subtle and may also be due to the presence of the catheter itself as opposed to infection [26]. If suspected, analysis of the urine may show the presence of white blood cells (WBCs). A high WBC count of >10 per µl is indicative of infection. A urine culture with counts of >10³ cfu/mL of a predominant pathogen will determine the type of bacteria present and susceptibility tests will determine the appropriate antibiotic for treatment. If left untreated, pylenephritis, calculus formation, bacteraemia, urosepsis and even death can result [53].

1.6.3. Treatment

For all patients on LTC, it is recommended that their catheters be changed according to their clinical needs or as recommended by the manufacturer of the catheter which is usually at 10 - 12 week intervals [21]. If symptomatic CAUTI is confirmed, evidence indicates that it is better to change the catheter before antibiotic treatment is initiated [16]. Infection with one type of organism can be treated with either trimethoprim, which acts upon most uropathogens except for *Pseudomonas* and *Enterococcus* species, fluoroquinolones which are effective against many Gram negative organisms including most *Pseudomonas* and *Proteus* species or with nitrofurantoin, a UTI - specific antibiotic that is effective against most uropathogens except *Pseudomonas* and *Proteus* species [53]. Patients with infections that are
polymicrobial may be treated with trimethoprim or a cephalosporin such as cefuroxime [53]. Seriously ill patients may require a two-drug treatment regimen to control infection, especially if they have repeated polymicrobial infections. For certain symptomatic patients, due to the high relapse rate, catheter replacement may have to be performed as often as every 2-3 weeks.

Infections associated with biofilms are rarely resolved using antibiotics alone even when the patient’s immune system is fully functioning [27]. Even if a symptomatic patient’s catheter is replaced and antibiotics given, there is a high incidence of recurrent infection causing severe distress and long-term morbidity to the patient [38]. It has been known for P. mirabilis infections of a single genotype to persist in the urinary tract despite many catheter changes, antibiotic treatment and periods of non-catheterisation [24]. Universally the only recommended guidelines to reduce the risk of bacterial infection are to maintain a closed drainage system and to minimise the duration of catheter usage as much as possible [53]. Despite the treatment measures, for some patients there is no satisfactory resolution to the problem and there remains a need to develop a strategy/measure to reduce the occurrence of CAUTIs.

1.7. Reducing CAUTIs

Little has changed in the way Foley catheters are designed other than the change from an open drainage system to a closed one which dramatically reduced CAUTIs. The correct size of catheter, proper aseptic technique upon catheter insertion and post-catheterisation care are important measures to limit the chance of acquiring an infection. The isolation of catheterised patients in some hospital settings is also
recommended to help prevent cross infection along with continued good hand washing practice. The overuse of catheters in particular needs to be carefully monitored and alternatives such as suprapubic and intermittent catheterisation should be considered as a means to reducing CAUTIs.

In the past irrigating the bladder with antimicrobial agents, instilling antimicrobial solutions into the drainage bag and soaking the catheter in an antiseptic prior to insertion have been studied as ways of reducing CAUTIs [68], [69]. These methods have proved unsuccessful and in cases with continuous exposure to anti - infective irrigating fluid an increase in resistant microorganisms has been shown [69]. A newer approach has involved examining the effect instilling aganocides into the bladder has on reducing urinary catheter blockage, encrustation and infection. Aganocides mimic the body’s own natural defences against infection. The aganocide NVC-422 is a derivative of the body’s natural biocide N-chlorotaurine which mimics the activity of pathogen - killing neutrophils [70]. Studies involving the instillation of NVC-422 into the bladder for 1 hour before being drained away showed it to possess broad spectrum antimicrobial activity against uropathogens, bacteria resistant to antibiotics, and bacteria encased in a biofilm [70]. As NVC-422 mimics the body’s own natural defences, bacterial resistance to it is thought to be low. NovaBay Pharmaceuticals are currently in the process of undergoing Phase 2 clinical trials on the use of NVC-422 solutions to control bacteria that develop on urinary catheters and in the bladder and if a positive reduction in CAUTIs and blockage is shown it is possible that installation of aganocide into the bladder could become a routine part of catheter care.
Jones et al (2006) [21] investigated loading the antimicrobial agent triclosan into catheter retention balloons to determine whether the membrane of the balloon could act as a barrier to control the release of the agent into the urine over a prolonged period. Catheterised bladder models infected with *P. mirabilis* revealed that triclosan diffused through the balloon and into the urine which allowed the catheters to drain freely for the experimental period of 7 days with little sign of encrustation. It was estimated that the retention balloon could give protective activity at a rate that was estimated to cover at least 12 weeks. The group did, however, highlight the importance of monitoring urinary flora for signs of the emergence of resistance to triclosan and this should always be considered when introducing antimicrobial agents to deal with infections.

The potential of bacteriophage (viruses that selectively infect bacteria) therapy in reducing/treating CAUTIs is also a strategy that has been studied. Carson et al (2010) [71] found that Foley catheters coated in a hydrogel treated with a cocktail of bacteriophage cultures were capable of not only preventing biofilm formation by bacteria commonly found in CAUTIs but was also able to kill off >99.9% of an established *E. coli* biofilm. Similarly Curtin et al (2006) [72], report on a hydrogel catheter coated in *Staphylococcus epidermidis* phage which significantly reduced biofilm counts of *S. epidermidis* by >4 logs over 24 hours. There are, however, important factors which should considered when evaluating the potential of phage to control biofilms. The high specificity of phage, bacterial mutants which exhibit phage resistance, how a patient’s immune system will respond to phage, the purity of phage and potential for transfer of genes encoding bacterial toxins or other virulence factors are areas of concern over their use [73]. A mixture of phage or engineered
phage may however help overcome these potential drawbacks. However, for urinary catheter biofilms, careful selection of phage would be required as a variety of bacterial strains may potentially colonise the catheter. The capacity of the catheter coating to adsorb phage, ensure their stability and infectivity and the impact of the presence of a conditioning film on the ability of phage to kill bacteria, require investigations but with further validation the approach may show some potential in reducing biofilms on medical devices.

Perni et al (2009) [74] reported on silicone elastomers containing a light - activating antimicrobial agent (LAAA) - methylene blue (MB) with and without gold nanoparticles (AU). Light of certain wavelengths transforms MB to an active state and in the process produces reactive oxygen species (ROS) [74]. ROS cause damage to a range of microbial components and thereby reduces the opportunity for the organism to develop resistance [75]. Polymers containing MB with AU showed a significant reduction in \textit{E.coli} and MRSA within five minutes of irradiation [74]. It is envisaged that short pulses of laser irradiation at the catheter entry site and along the catheter length would be sufficient to prevent the accumulation of bacteria and would hence reduce the incidence of catheter - related infections. The scale - up of this to a commercial catheter system is critical but could prove to be beneficial in reducing CAUTIs. Similarly the incorporation of the photosensitiser toluidine blue (TBO) into polymers such as polyurethane and silicone have exhibited exceptional kill against MRSA and \textit{E.coli} [75].
A more common approach to prevent bacterial colonisation on urinary catheters and subsequent biofilm formation has involved loading catheters with broad spectrum antimicrobial agents.

1.7.1. Means of Making Catheters Antimicrobial

There are a variety of ways in which catheter materials can be made antimicrobial and are detailed below.

1.7.1.1. Immersion/Dipping

Simply immersing or dipping the material into an aqueous antibiotic/antiseptic solution forms a protective anti-infective layer over the material. A recent example of this by Hernandez et al (2009) [76] involved dipping a polymer into a combination of N-acetylcysteine, gentamicin and amphotericin B for the prevention of microbial colonisation of ventricular assist devices. The antimicrobials adsorb to the surface but are not dispersed throughout the polymer matrix, making the drugs wash off easily. This may be sufficient to reduce early onset bacterial colonisation but is not suitable for preventing colonisation upon devices that are used long-term [77]. It has been reported that immersion of hydrophobic materials into an antimicrobial solution results in more weak and limited surface attachment compared to hydrophilic materials [77]. Applying hydrogels to catheters makes them more hydrophilic and also helps in the insertion process by reducing trauma caused the urethral mucosa. As a consequence of their high water content, antimicrobial agents are easily taken up and released but their effectiveness with regards to CAUTIs has primarily been studied over a short duration (<7 days) [78].
1.7.1.2. Coating

Coating polymers with antimicrobial agents is and continues to be a much researched area both for use in urinary catheters and other catheter-related devices. Coatings are usually formed on the external surfaces of catheters. The nitrofurazone-coated urinary catheter, however, is an example where both the external and internal surfaces of the catheter are coated [58]. Nitrofurazone is applied in a silicone coating onto the catheter surfaces which releases into the surroundings. Coatings can be added to polymers post-manufacture which has its benefits but like materials that are immersed/dipped into antimicrobial solutions, coatings of antimicrobial agents are easily washed off once inserted into the body making their duration of protective activity suitable only for short-term use. The formation of a conditioning film on the surface can also result in the coating being masked affecting the release of antimicrobial agents.

1.7.1.3. Matrix Loading

Matrix loading is a further technology used whereby antimicrobials become directly incorporated into the polymer matrix either by an admixture or impregnation process. This can be performed at the polymer synthesis stage or post-manufacture. The admixture process involves mixing the antimicrobial powders into the raw material during the pre-manufacture stage. A polymer in which the drugs are distributed throughout the whole of the device in the form of small drug particles is created. An example of this technology is the External Ventricular Drain (EVD) (Ventriclear II) [79] used in neurosurgery and the Central Venous Catheter (CVC) [80] which both contain an admixture of minocycline and rifampicin. Similarly antimicrobial agents can be distributed through the whole polymer protecting the internal and external
parts of the catheter, by using an impregnation process [81]. Impregnation is a solvent-based procedure that introduces the antimicrobial agents into the polymer matrix as molecules [81]. A successful application of this is the Bactiseal Shunt used in neurosurgery that contains rifampicin and clindamycin [82-84]. Neither the admixture nor the impregnation process result in the rapid wash off of the antimicrobials, instead the agents are continually and gradually released over time. The impregnation process also has the added benefit of the drugs being in a molecular form giving a more even distribution and release from the polymer compared to the crystalline admixture polymers. Although the Ventriclear EVD and Bactiseal Shunts are designed to release antibiotics short-term there may be scope for matrix loaded devices to be effective over a longer duration.

1.7.2. ReleaseNF® Urinary Catheter

Currently there are two main antimicrobial urinary catheters on the market. The ReleaseNF® catheter (Rochester Medical Corporation, Minnesota, USA) (Figure 1.6) and the Bardex® I.C. catheter (Bard Medical, Georgia, USA) (Figure 1.7). The ReleaseNF® catheter contains the urinary antibiotic nitrofurazone which is matrixed into the silicone of the catheter’s inner and outer surface allowing the antibiotic to elute both intraluminally and extraluminally [85]. Nitrofurazone is a broad spectrum agent that is claimed to be effective against many organisms associated with CAUTIs and the catheter works by eluting the nitrofurazone into the urethral tract and killing any planktonic bacteria before they get chance to colonise the catheter and develop into a biofilm [85].
1.7.2.1. Review of the ReleaseNF® Urinary Catheter Clinical Trials

There have been several clinical trials evaluating the effectiveness of the ReleaseNF® catheter on reducing CAUTIs. Maki et al (1997) [86] conducted a prospective double blind trial involving 344 patients and found a three-fold reduction in the rate of CAUTI using the nitrofurazone catheters compared to conventional silicone catheters (2.4% vs 6.9%) which was significant for the first 5 days of use. Leclair et al (2000) [87] reported on the effect of the nitrofurazone catheters in a non-randomized surveillance study involving 30 patients on burns intensive care units. A baseline incidence of CAUTI in the burns unit was reported as being 24.4 infections per 1000 catheter days. The introduction of the nitrofurazone catheter to the unit showed a significant reduction to 7.3 infections per 1000 catheter days. Lee et al (2004) [88], reviewed 177 patients from five university hospital settings and also found a significantly lower number of infections using the
nitrofurazone catheter between days 5 - 7. Stensballe et al (2008) [89], reported on a double blind randomized controlled trial involving 212 trauma patients. They found the incidence of catheter-associated bacteriuria/funguria to be significantly less in the nitrofurazone group (13.8/1000 catheter days compared to 38.6/1000 catheter days in the control group). From the examples of the clinical trials given and the many more, overall the clinical data imply that the nitrofurazone-coated urinary catheters are effective in reducing the rate of CAUTIs in patients on short-term catheterisation.

1.7.3. Bardex I.C.® Urinary Catheter

The second of the main antimicrobial urinary catheters on the market is the Bardex I.C.® catheter which is coated on its inner and outer surface with a proprietary silver alloy. It has a layer of hydrogel on the outside, a gold and palladium layer that claims to mediate the slow sustained release of the silver ions and keeps the silver surface stable, and the silver itself which is chemically anchored to the catheter surface and is released to minimise microbial adherence and biofilm formation [90].
1.7.3.1. Silver as an Antimicrobial Agent

As silver conveys strong antimicrobial activity to a broad spectrum of organisms even at low concentrations, silver-based compounds are used in many medical applications. Various forms of silver e.g. metallic silver, ionic silver, and silver nanoparticles have been employed to decrease infections associated with burns as well as in the prevention of bacterial colonisation upon numerous medical devices including catheters [91]. It has even been used within textiles and deodorants to eliminate bacteria. The vast majority of silver antimicrobial activity is attributable to the action of the silver ion (Ag⁺) [92]. Due to the increase in multidrug-resistant bacteria against conventional antibiotic treatment, there has seen a resurgence in the use of silver [93].
Until recently little has been known about the mechanisms silver uses to kill microbes and these are still only partially understood [94]. It is known that silver has an ability to attack multiple sites within a cell which has benefits in the protection against resistance [92]. Silver ions bind strongly to electron donor groups containing sulphur, oxygen and nitrogen and interact with thiol groups of bacterial proteins and enzymes [93, 95, 96]. It inhibits bacterial growth, cell wall division and causes structural changes to cell membranes and contents within the cell [94, 95, 97]. Once inside the cell the silver ions interfere with DNA functions and cause suppression of processes such as respiration and electron transport components ultimately leading to cell death [93, 95]. There is still some scepticism as to whether bacteria can become resistant to silver. The lack of standardised methods to determine bacterial susceptibility to silver and absence of recognised breakpoints complicates the interpretation of silver susceptibilities and resistance. A few papers have however reported on silver - resistant bacteria found in environments where silver usage is greatest [98, 99]. Plasmid mediated silver resistance has been identified in some bacteria and in one case the silver resistant plasmid isolated from a Salmonella strain resulted in the deaths of several patients on a burns unit [96, 99]. The probability of transfer of silver resistance genes is considered to be low and the contribution to the emergence of antibiotic resistant bacteria is unclear [96]. Whether silver will cause resistance problems in the clinical setting or remain a rare occurrence is uncertain but it is important to monitor because modern day technology has developed a wide range of products that depend on silver as a key microbicidal component [98].
1.7.3.2. **Review of the Bardex I.C.® Urinary Catheter Clinical Trials**

Numerous clinical trials have taken place to determine how effective the silver alloy-coated urinary catheters are in reducing CAUTIs. To highlight a few, Verleyen *et al* (1999) [100], performed a prospective randomized trial involving 180 patients on general and medical surgical wards and reported that by day 5 there was a significantly lower (p < 0.003) percentage of patients (6.3%) with the silver alloy catheter whom developed a CAUTI compared to (11.9%) the control latex catheter group. Newton *et al* (2002) [101] reported on a retrospective trial where they took a baseline rate of CAUTI in burns patients who where catheterised between the years of 1998 and 1999. Upon the introduction of the silver alloy catheter from the year 2000 over the same length of time they found there to be a significantly lower (p = 0.029) incidence of CAUTI. A meta-analysis conducted by Schumm and Lam (2010) [5], also suggests that the use of silver alloy indwelling catheters for catheterising hospitalised adults short-term reduces the risk of catheter associated bacteriuria. Overall the clinical data implies that the silver alloy-coated catheter is effective in reducing the rate of CAUTIs in patients on short-term catheterisation.

1.7.4. **Limitations**

There are however limitations to the studies involving the nitrofurazone and silver alloy-coated urinary catheters. Poor quality of available evidence, small study sizes and heterogeneity, unclear randomization and blinding, wide variation in antibiotic usage, absence of data on clinically meaningful endpoints and extensive post-randomization exclusions are amongst the limitations. Patients having asymptomatic bacteriuria were commonly used as an outcome but the definition between studies
varied and no reference was made to distinguishing between symptomatic and asymptomatic bacteriuria (both were called UTI) [5]. Traunter et al (2004) [26] argued that the presence of bacteria in the urine in otherwise asymptomatic catheterised patients does not necessarily indicate that invasive infection of the urinary tract has taken place. Further well - designed trials with clear aims, definitions and outcomes are needed to clarify the comparative utility and economic value of these types of antimicrobial urinary catheters [102].

1.7.5. Other Antimicrobial Urinary Catheters

Besides the two marketed antimicrobial urinary catheters, several other antimicrobial agents have been incorporated into catheters and investigated for their ability to reduce CAUTIs. A large randomized clinical trial by Riley et al (1995) [103] involving 1309 patients on indwelling catheterisation found that 11.4% of patients who had a silver oxide catheter in place developed bacteriuria compared to 12.9% of patients in the control group, this was not statistically significant (p = 0.45). Similarly a meta - analysis conducted by Saint et al (1998) [104] reported on clinical studies, four of which involved the silver alloy - coated urinary catheter [105-108] which had a significant effect on reducing CAUTIs as previously stated and four involving the silver oxide urinary catheter [103, 109-111] which did not significantly reduce CAUTIs and has since been taken off the market.

Further to this Gaonkar et al (2003) [112], impregnated urinary catheters on the outside with chlorhexidine/triclosan and/or chlorhexidine/silver sulfadiazine/triclosan (latex and silicone) and monitored the ability of bacteria to adhere to the extraluminal surface of the catheter in an in - vitro model. The chlorhexidine/silver
sulfadiazine/triclosan silicone catheters were found to resist colonisation by *S.aureus* and *S.epidermidis* for 23 and 24 days respectively. Cho *et al* (2003) [113] showed that urethral catheters prepared by dipping with poly ethylene-co-vinyl acetate (EVA) and EVA/poly ethylene oxide (PEO) blends containing gentamicin exhibited antibacterial activity for 7 days against *P.vulgaris, S.aureus* and *S.epidermidis*. Similarly norfloxacin - releasing urethral catheters prepared by dipping with EVA and PEO and poly(dimethyl siloxane) (PDMS) as above were found to release norfloxacin for 30 days with the growth of *E.coli, K.pneumoniae* and *P.vulgaris* being inhibited for 10 days [114]. In addition to antimicrobial catheters, urinary catheters which are designed to be non - stick such as those with coatings consisting of polyethylene glycol (PEG) sidechains have also been investigated and have displayed a good ability to resist microbial attachment [115].

Despite the countless studies that have looked into developing biofilm - resistant materials, with some good *in - vitro, in - vivo* and clinical evidence of biofilm inhibition, nothing has led to a urinary catheter or treatment that has been considered a true success in reducing CAUTI in patient on LTC.
1.8. Release Mechanisms of Antimicrobials from Polymers

The mode in which antimicrobial agents are incorporated into devices can affect the mechanism in which the agents release. For example, drug release may involve diffusion, dissolution, erosion or swelling. Matrix type devices, in which the antimicrobial agent is dispersed in a carrier e.g. a polymer, usually have some degree of mobility and may release by a diffusion process. Once the polymer containing the agent comes into contact with an aqueous environment, drug molecules diffuse to the interfacial region of the polymer and migrate from it to the external environment. This is usually a result from differences in the concentration gradient. Agents may also be triggered to release due to the ingress of an environmental agent e.g. water into a matrix, which can effectively act to swell the system and increase the polymer mesh size. The aqueous content within the formulation enables the drug to diffuse through the swollen network to the external environment. Alternatively, the event could lead to a chemical reaction which unbinds the agent e.g. hydrolysis or plasticise the matrix which allows physically bound molecules to diffuse out of the system [116]. A further drug release mechanism more common in tablet matrix systems is the process of dissolution where the drug is embedded in a slowly dissolving substance i.e. the drug particles are coated or encapsulated with slowly dissolving materials like cellulose [116, 117]. In the early stages of dissolution when the polymer comes into contact with a fluid environment, the polymer and drug may swell before dissolution starts and drugs start to exert their effects. With erodible devices the agents are incorporated into a carrier which becomes eroded away by the environment through physical processes such as dissolution or chemical processes such as hydrolysis of the polymer backbone or crosslink [116, 117].
Thus far, the mainstay of anti-infective urinary catheters has focused the delivery of drugs over a short duration and clinical trials on long-term use are limited. Extrapolation of short-term studies to those catheterised long-term is unwise. A study by Kohnen et al (2003) [118] produced a ventricular catheter by impregnating it with rifampicin and sparflaxacin using the solvent-based impregnation process. They report the catheter in *in vitro* experiments showed a 99.8% reduction in the colonisation of *S.epidermidis* compared to control material which was effective for at least one year. The successful clinical trial results achieved through the use of the Bactiseal Shunt [82-84] and the potential of this impregnation technology as reported by Kohnen *et al* (2003) [118] for extending the duration of activity has lead to the possibility of using this processing technology to see if it could be implemented to prevent bacterial colonisation on urinary catheters in patients on LTC.
1.9. AIMS

Biomaterials used to produce urinary catheters for long-term use that are completely resistant to bacterial colonisation for clinically significant periods remain elusive. This project proposes to modify a silicone urinary catheter by impregnating it with a suitable combination and concentration of antimicrobial agents intended to provide protection against bacterial colonisation and subsequent biofilm development in patients that are on LTC. Patients on LTC will require a catheter to deliver a long duration of protective activity covering a broad array of uropathogens without the development of bacterial resistance if the rates of CAUTIs are to be reduced.

The thesis takes a multidisciplinary approach and reports on the manufacture, development and rigorous in-vitro testing of the antimicrobial urinary catheter. In addition to the ability of the antimicrobial agents to inhibit bacterial colonisation (Chapters 2 and 3), antimicrobial content and release profiles (Chapter 4), the effect of processing on the catheter surface (Chapter 5), and mechanical properties (Chapter 6) will be elucidated in order to achieve the desired properties of the product.
CHAPTER 2
SCREENING TESTS
2.0. SCREENING TESTS

2.1. INTRODUCTION

2.1.1. Requirements of an Antimicrobial Urinary Catheter

The main requirements which must be considered in the development of an antimicrobial biomaterial for use as a long-term indwelling urinary catheter are that it should be effective against principal pathogens involved in CAUTIs over a long duration typically the whole period of catheter implantation (10 - 12 weeks). To inhibit bacterial colonisation, protection must cover both the inner and outer surfaces of the catheter and the antimicrobial agents used to achieve this must have a clean clinical history with no known significant risk of toxicity. The drugs should remain stable upon sterilisation and storage and the incorporation of them into the silicone must not adversely affect the mechanical performance of the catheter or balloon. The selection of antimicrobial agents and their concentrations are most important to prevent the emergence of bacterial resistance. The catheter is not intended to be capable of preventing bacterial attachment but rather to prevent colonisation and subsequent biofilm formation which make treatment more difficult.

2.1.2. Method of Antimicrobial Delivery

A controlled release of a continuous delivery of sufficient doses of antimicrobial agents over a prolonged period is required if the antimicrobial catheter is to show any potential in reducing CAUTIs. Ideally the drugs must be evenly dispersed throughout the polymer matrix but produce an initial burst effect to reduce the risk of infection upon catheter insertion followed by a long period of slow sustained release
to inhibit later infection. The matrix loaded impregnation process is a suitable method for incorporating agents in this way. A long duration of activity without the drugs being immediately released and incorporation throughout the whole of the material providing inner and outer surface protection can be provided using this process. The impregnation process [81] requires the antimicrobial agents to be dissolved in a solvent e.g. chloroform. Upon immersion of the silicone material, the chloroform acts to swell the matrix and the antimicrobial agents become incorporated through the whole of the material at a molecular level. Once the chloroform is removed the silicone returns to its original shape and size leaving the antimicrobial agents within the matrix. The main limitations of the impregnation process are that not all antimicrobial agents readily dissolve in chloroform and not all agents combine well.

### 2.1.3. Antimicrobial Agent Selection

Several criteria need to be taken into account when selecting antibiotics for incorporation into silicone materials. The agents need to be capable of molecular migration through the cross linked silicone polymer [81] and due to the possibility of bacteria becoming resistant if the catheter contains only one drug, a combination of antimicrobial agents is required to reduce this risk, the objective being that at least two if not three drugs with different mechanisms could act on Gram positive and Gram negative invading organisms at any one time.

The antimicrobial agents investigated in this project are listed in Table 2.1 and those selected for further testing are highlighted.
Table 2.1: Antimicrobial agents and their properties [119-121]. (agents highlighted in red were selected for further testing)

<table>
<thead>
<tr>
<th>Class/drug</th>
<th>Molecular weight (g/mol)</th>
<th>Solubility in water</th>
<th>Solubility in organic solvents</th>
<th>Antibacterial spectrum</th>
<th>Reason for rejection or acceptance of agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminoglycosides</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gentamicin sulphate</td>
<td>477.6</td>
<td>Highly soluble</td>
<td>Low: alcohol, acetone, CHCl₃, ether, benzene</td>
<td>Broad spectrum, many Gram positive and Gram negative bacteria</td>
<td>Not dissolvable in CHCl₃</td>
</tr>
<tr>
<td>Lincosamides</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clindamycin hydrochloride</td>
<td>424.9</td>
<td>Highly soluble</td>
<td>High: DMF, EtOH, MeOH, CHCl₃, ether, benzene Low: acetone</td>
<td>Mostly active on Gram positive bacteria</td>
<td>Good Gram positive activity but not compatible with some other agents and expensive</td>
</tr>
<tr>
<td>Quinolones</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>319.3</td>
<td>Slightly soluble</td>
<td>High: acetic acid, CHCl₃, Slight: alcohol, acetone, ethyl acetate Low: ether</td>
<td>More effective against Gram negative than Gram positive bacteria but covers several important pathogens in both groups</td>
<td>Good Gram negative activity but sparfloxacin gave longer duration of activity</td>
</tr>
<tr>
<td>Sparfloxacin</td>
<td>392.4</td>
<td>Practically insoluble</td>
<td>High: acetic acid, CHCl₃, NaOH Slight: EtOH Low: ether</td>
<td>More effective against Gram negative than Gram positive bacteria but covers several important pathogens in both groups</td>
<td>Long duration of activity particularly towards Gram negatives</td>
</tr>
<tr>
<td>Rifamycins</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rifampicin</td>
<td>823.0</td>
<td>Slightly soluble</td>
<td>High: DMSO, THF, CHCl₃, MeOH, ethyl acetate Low: acetone</td>
<td>Mostly Gram positive bacteria and some fastidious Gram negatives. Mycobacteria</td>
<td>Long lasting Gram positive (staphylococcal) activity</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minocycline</td>
<td>457.5</td>
<td>Soluble</td>
<td>High: alkali hydroxides and carbonates Low: EtOH, CHCl₃, ether</td>
<td>Broad spectrum, many Gram positive and Gram negative bacteria</td>
<td>Not dissolvable in CHCl₃</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>290.3</td>
<td>Slightly soluble</td>
<td>High: benzyl alcohol Slight: alcohol, acetone, CHCl₃ Low: ether</td>
<td>Mostly Gram negative bacteria but with some Gram positive activity</td>
<td>Good Gram negative activity but commonly used to treat UTIs and showed signs of resistance</td>
</tr>
</tbody>
</table>
### Antiseptics

<table>
<thead>
<tr>
<th>Antiseptic</th>
<th>Molecular Weight</th>
<th>Solubility</th>
<th>Solvent</th>
<th>Spectrum</th>
<th>Activity Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triclosan</td>
<td>289.5</td>
<td>Practically insoluble</td>
<td>High: EtOH, MeOH, acetone CHCl₃ Low: water</td>
<td>Broad spectrum, many Gram positive and Gram negative bacteria (except Pseudomonads), some antifungal activity</td>
<td>Long duration of activity against a broad spectrum of bacteria</td>
</tr>
<tr>
<td>Hexetidine</td>
<td>339.6</td>
<td>Slightly soluble</td>
<td>High: acetone, alcohol, CHCl₃, dichloromethane</td>
<td>Broad spectrum, many Gram positive and Gram negative bacteria</td>
<td>Short duration of activity even at higher concentrations</td>
</tr>
<tr>
<td>Octenidine dihydrochloride</td>
<td>623.8</td>
<td>Slightly soluble</td>
<td>High: EtOH, MeOH, CHCl₃</td>
<td>Broad spectrum, many Gram positive and Gram negative bacteria</td>
<td>Short duration of activity even at higher concentrations</td>
</tr>
</tbody>
</table>

#### 2.1.4. Resistance Theory and Approach

The increasing problem of bacterial resistance in healthcare settings is a worldwide issue, hence the potential for the development of resistance to antimicrobial agents introduced into polymers for clinical use is of concern [77]. Antibiotics can be classified according to their minimum inhibitory concentration (MIC). This refers to their ability to inhibit the growth of susceptible bacteria. Antibiotics released at sub-cidal concentrations may evoke selection of resistant strains and intensify infectious complications [119]. This can depend on the concentration of antibiotic, duration of action, size of the inoculum and frequency of resistant variants.

An antibiotic concentration window as shown in Figure 2.1 exists in which resistant mutants are selectively amplified [122]. The lower boundary is the drug concentration at which growth inhibition of the majority of the susceptible cells begins, and is approximated by the MIC [122]. At a concentration just above the MIC, only one mutation is required for a bacterial cell to grow in the presence of the antibiotic and an infection can contain sufficient cells for first step resistant mutants to be present. It has been proposed that the upper boundary of the window acts as...
antibiotic concentration that blocks the growth of first step resistant mutants and requires cells to attain two resistance mutations in order to grow [122]. Events of this type are rare and it is expected that mutants will not be recovered above this concentration. This is termed the mutant prevention concentration (MPC) [122]. Resistant mutants are therefore selectively enriched in the concentration range between the MIC and the MPC. This is designated the mutant selection window (MSW) [122].

**Figure 2.1:** A pharmacodynamic depiction of the mutant selection window [123].

MPC = Mutant prevention concentration. MIC = Minimum inhibitory concentration
C<sub>max</sub> = Maximum concentration
Each type of bacterial - antimicrobial combination has its own characteristic MSW. Resistant mutants can also be enriched by repeated passage of cells at concentrations just below the MIC [123]. Low drug concentrations do not enrich resistant mutants per se but allow for bacterial populations to increase which consequently indirectly fosters the generation of new mutants that will be enriched by subsequent antimicrobial challenge. If antibiotic treatment were continued, resistant mutants would be selectively enriched and treatment may fail. Passage of a pathogen through many treated patients is expected to gradually increase the mutant fraction of the bacterial population [123]. Antimicrobials may cure the vast majority of infections but when millions of cells are considered the development of resistance is an inevitable consequence of dosing strategies that place drug concentrations inside the MSW, especially if treatment is prolonged [123].

If the MSW can be made narrower or closed, no mutant will be selected. One of the ways to achieve this is to avoid prolonged therapy within the MSW concentration range as mutant fractions will be enriched [122], but this is difficult to achieve for patients undergoing urinary catheterisation as certain groups require long - term therapy. Dual drug therapy involving a combination with two or more different classes of drugs released at concentrations above their MICs should require two resistance mutations for growth. This can close the mutant selection window if their normalised pharmacokinetic profiles superimpose at concentrations that inhibit growth [123]. When antibiotic concentrations drop below the MIC both mutants and wild type cells increase in numbers and more spontaneous mutants are generated. If treatment is stopped before mutants reach a high number they may not be detected. For mutants to be selected, the concentration of one compound must occasionally fall
below its MIC while that of the other remains above its MIC but below its MPC. The MSW is open for the drug that remains above its MIC for resistance to that compound to be enriched. After a population resistant to one compound has grown out, selection of resistance to the second compound soon follows when its concentration approximates the MIC [122].

It has been demonstrated that rifampicin at concentrations higher than its MIC can select for resistant staphylococci if applied to a large inoculum but at sub-inhibitory concentrations and if applied to a smaller inoculum, rifampicin resistance may not be enhanced [124]. The risk for selection of resistant microorganisms is therefore the period at which the concentration of antibiotics approximates to the MIC or when it is lower than its value [124]. The longer the concentration of antibiotic is below the MIC the greater the risk that resistant organisms will be selected. An example of where the use of a combination of antimicrobials has been successful in acting to decrease the selection for resistant organisms is in the drugs prescribed for the treatment of tuberculosis. It is therefore important to try to create a combination of antimicrobial agents that release equally from the biomaterial at concentrations over prolonged periods that are above their MIC towards the uropathogens if resistance is to be avoided. It is anticipated that the antimicrobial agents selected for further investigation, combined would have the potential to prevent the emergence of bacterial resistance. As rifampicin is active on Gram positive organisms, sparfloxacin predominantly on Gram negative organisms and triclosan on both, if bacteria are sensitive to two or more of the agents then this could provide a dual drug type therapy that could help in the protection from resistance.
2.1.5. Selected Antimicrobial Agents

Below is a description of the antimicrobial agents selected from Table 2.1 to impregnate into the urinary catheter. Further reasoning for the selection of these agents is given in Section 2.4.

2.1.5.1. Rifampicin

Rifampicin is a bactericidal antibiotic belonging to the rifamycin group (Figure 2.2). It acts by inhibiting RNA polymerase by binding to its β-subunit thereby preventing the transcription to mRNA and subsequent translation to polypeptides and proteins [125]. It is used to treat a variety of bacterial infections especially those due to organisms that are Gram positive. Rifampicin is well tolerated and a typical oral dose consists of 150 - 300 mg. Excretion via faeces i.e. bile accounts for 60 - 65% with the remaining being excreted via the urine. Rifampicin is a solid red coloured antibiotic and can impart a harmless red/orange colour to urine and to a lesser extent, tears and sweat. Bacterial resistance to rifampicin is common since it only requires a single mutation step to render them resistant hence it is normal to use rifampicin in combination with other drugs that act synergistically to improve outcomes of infection [15].
2.1.5.2. Sparfloxacin

Sparfloxacin is a member of the quinolone family. Quinolones are chemotherapeutic bactericidal drugs which can be further divided into the subset fluoroquinolones, to which sparfloxacin belongs. It has a fluorine atom attached to its central ring system at the C6-C8 position (Figure 2.3) and possesses a broad range of anti - Gram positive and anti - Gram negative activity [127]. It acts by interfering with DNA gyrase or topoisomerase enzyme types II and IV thereby inhibiting DNA replication and transcription [127]. DNA gyrase is the target in many Gram negative organisms and topoisomerase IV enzyme the target in many Gram positive organisms [127]. Sparfloxacin has been used in the past to treat respiratory disorders. A typical oral dose is 400 mg once daily on the first day, reducing to 200 mg per day thereafter [127]. Elimination of sparfloxacin is through the renal, biliary and transintestinal secretion routes [128]. Fluoroquinolones are quite well tolerated with most side effects involving the gasterointestinal and central nervous system being mild to moderate. Photosensitivity has been shown to be prevalent amongst all quinolone antibiotics and this is especially true of sparfloxacin [129] with reactions such as
erythema and rashes being reported [127]. Adverse events are unusual, but quinolones have been associated with problems of the cardiovascular system such as prolongation of the QT interval and of the muscoskeletal system such as tendinitis and tendon rupture in particular being more common amongst the elderly and athletes in training who are receiving corticosteroids [130]. Quinolones may adversely interact with anticoagulants e.g. warfarin and medications for seizures e.g. epilepsy medications and should be used judiciously to minimise complications as *Clostridium difficile* associated diarrhoea [130]. Resistance to quinolones can occur via three mechanisms: efflux pumps, plasmid mediated or mutations at sites within DNA gyrase and topoisomerase IV enzyme which can decrease the binding affinity to quinolones and their effectiveness [131].

![Figure 2.3: Structural representation of sparfloxacin [132].](image-url)
2.1.5.3. Triclosan

Triclosan (2,4,4’-trichloro-2’-hydroxydiphenylether) belongs to a class of compounds known as the bisphenols (Figure 2.4). It has a broad spectrum of activity acting mostly as an antibacterial but possesses some antifungal and antiviral activity. Triclosan acts on the fatty acid biosynthetic pathway. It binds to the enoyl-acyl carrier protein reductase enzyme (ENR) which is coded by the Fab1 gene [133]. Binding to this increases the enzyme’s affinity for nicotinamide adenine dinucleotide (NAD$^+$) and by interacting with amino acid residues of the enzyme’s active site, a stable ternary complex of ENR-NAD$^+$ is formed which cannot participate in fatty acid synthesis [133, 134]. Fatty acids are components of phospholipids and sphingolipids that make up cell membranes which are essential for microbial systems [135].

\[
\text{Figure 2.4: Structural representation of triclosan [136].}
\]
Triclosan has been in use for more than 40 years and can be found in many products including surgical scrubs, deodorants, hand soaps, toothpastes as well as in the fabric/plastics industries, for example having been incorporated into chopping boards [137]. It is not used as a systemic antimicrobial agent but is found in clinical use, for example in antibacterial sutures (coated polyglactin 910 suture containing triclosan) [138]. Triclosan has a favourable safety profile. Toxicology studies show that it is not a carcinogen, mutagen or teratogen and has been found to be safe in reproductive studies [135, 139, 140]. Recently however the U.S. Food and Drug Administration (FDA) has raised concerns on the overuse of triclosan, its efficacy in household products and conflicting studies regarding links between triclosan and adverse health effects in animals and its uncertainty over potential harmful effects in humans [141, 142]. Questions have been raised as to whether triclosan disrupts the body’s endocrine system which regulates growth and development and if the overuse of triclosan causes bacteria to become more resistant to antibiotics. Bacteria develop resistance to triclosan using a variety of mechanisms. These include target mutations, increased target expression, active efflux and enzymatic inactivation or degradation [134, 143]. Certain bacteria like *P. aeruginosa* have innate resistance to triclosan as they possess efflux pumps that pump triclosan out of the cell. Due to the similar mechanisms of resistance to those seen with antibiotics, there is concern that biocides may share target sites with antibiotics used in clinical practice and bring about cross-resistance. This has in fact been shown in laboratory studies where a *Fab1* mutation in triclosan caused cross-resistance with other antibiotics in *E.coli* [144]. Whether its widespread use and potential overuse will cause a major increase in antibiotic resistance in a clinical setting is a subject of much debate. A regulatory
2.1.6. Laboratory Testing Methods

Before clinical trials commence, laboratory tests need to be undertaken to gain insight into the likely outcome of reducing infections in clinical situations. A number of techniques and models have been used to study the effectiveness of antimicrobial materials in reducing bacterial numbers, although there is no standard method.

A conventional screening system used is the agar diffusion assay which is based on the inhibition of microbial growth on an agar plate by the diffusible activity of the antimicrobial agent [146]. The size of the zone of inhibition (ZI) surrounding the test material is indicative of its antimicrobial activity. The method is, however, only applicable to those antimicrobial systems that give rise to diffusible activity and cannot be used when silver is the antimicrobial agent. The agar diffusion method can be adapted and by transferring the material onto freshly inoculated agar plates, the duration of antimicrobial activity can be examined [81]. This test is termed the serial plate transfer test (SPTT). It is a useful screening test for investigating the diffusibility of agents from polymers and to study their spectrum of antimicrobial activity as well as an indirect way of gauging the agent’s ability to provide a long duration of activity. A short SPTT duration can indicate activity is refined to the surface of the material whereas a more persistent duration indicates the antimicrobial agents diffuse from the matrix. Antimicrobial agents can be assessed on an individual basis or in combination. The SPTT can also help to detect for the report reviewing data indicating triclosan to be safe and the more recent conflicting data from the U.S FDA is due to be published in 2011 [145].
possibility of bacteria becoming resistant to the agents. As the drugs leach out from the material its repeated passage through lawns of bacteria can result in visible bacterial growth attached to the material or colonies within the ZI indicating possible resistance which can be examined further.

Historically, to assess the bactericidal activities of antimicrobial agents, time - kill studies have been used [147]. There are several ways of performing these studies but all basically involve exposing the material to a suspension of cells for a determined period of time to allow for the cells to attach. Non - adherent cells are removed by rinsing and the remaining adhered cells quantified [52]. It is important to assess the antimicrobial agents activity against attached bacteria as they can show a decrease in antimicrobial susceptibility compared to planktonic forms [148]. There are a number of ways of quantifying adhered bacteria, each with their own advantage for specific purposes. Examples include: microscopy techniques (light, image analysed epifluorescence, scanning electron microscopy) for counting and morphological observations of adherent bacteria, and viable bacterial counting techniques which can involve removal of the bacteria from the surface by sonication and quantifying them by colony counting on agar plates, radiolabelling or 5-cyano-2,3,-ditolyl tretazolium chloride (CTC) staining methods [52, 149]. Roll plate methods where bacteria attached to the material are rolled back and forth over an agar plate and bacterial growth observed have also been used [150]. A major disadvantage of this is that only the external surface can be cultured [151]. Ideally it is hoped that the antimicrobial agent release will be sufficient to kill 100% of attached bacteria. The test is termed the tK100 test and refers to the time taken to kill 100% of attached bacteria [152].
2.1.7. Objectives

It is a requirement of the antimicrobial urinary catheter to give a long duration of activity against pathogens commonly isolated from patients on LTC and to provide protection from bacterial colonisation and the development of resistance. This chapter describes the screening tests used to select the effectiveness of the chosen antimicrobial agent’s ability to provide a long duration of protective activity. It is expected that as rifampicin acts on Gram positive organisms, sparfloxacin predominantly on Gram negative organisms and triclosan on both, the combination of agents could also help reduce the opportunity of bacteria becoming resistant. The SPTT and tK100 tests were used to establish this.
2.2. METHODS

2.2.1. Biomaterial
Medical grade all silicone sheets (Goodfellow, Huntingdon, UK) of 1 mm thickness were cut to form 6 mm discs and used in the SPTT (see section 2.2.5) and tK100 (see section 2.2.6) tests. For Chapters 3 to 6, Simpla all - silicone (14 Fr, Male) Foley catheters supplied by Coloplast, Peterborough, UK were used for testing purposes.

2.2.2. Antimicrobial Agents
Rifampicin R3501 (Sigma-Aldrich, Poole, UK), Sparfloxacin 56968 (Sigma-Aldrich Poole, UK) and Triclosan USP (Ciba Speciality Chemicals, Macclesfield, UK) were the antimicrobial agents used predominantly through the course of the project.

2.2.3. Bacterial Characterisation
Clinical strains of bacteria isolated from the urine of patients with CAUTIs were obtained from the Microbiology Department at the Queen’s Medical Centre, Nottingham, UK. Organisms were received plated on Chromogenic UTI Medium (Oxoid, Basingstoke, UK). Chromogenic UTI Medium is a differential agar which provides presumptive identification of the main pathogens associated with CAUTIs as indicated by the colony colour (Table 2.2). Individual colonies were sub-cultured onto Blood Agar Base No.2 with sheep blood (SBA, Oxoid) or Cystine-Lactose-Electrolyte-Deficient Agar (CLED, Oxoid) as appropriate to obtain pure cultures. Organisms were fully characterised by the following standard microbiological techniques: Gram Stain (Pro-Lab Diagnostics, Cheshire, UK), catalase test (hydrogen peroxide - Thornton and Ross Ltd, Huddersfield, UK),
DNAse test (Oxoid) and oxidase test (Oxoid) as appropriate. API Staph or API 20E were carried out according to the manufacturer’s instructions (bioMérieux® SA, Marcy l’Etoile, France) and antibiograms (Oxoid) as shown in Table 2.3. Meticillin susceptibility was determined by growth on Iso-Sensitest Agar (ISA, Oxoid) by incorporating 4% Polyvinyl Pyrrolidone (Sigma-Aldrich, Dorset, UK) with or without meticillin at 12 µg/mL [153]. Precise MICs were obtained of rifampicin using E-Test strips (AB Biodisk, Solna, Sweden) and of sparfloxacin and triclosan using an agar incorporation method with a concentration ranging from 0.008 - 3 µg/mL as shown in Appendix 1. All organisms were stored in Cryobank vials (Mast Diagnostics, Merseyside, UK) and kept in a -80°C freezer for future use. Bacteria selected for this study were those most commonly isolated from the urine of patients with CAUTIs and which had an antimicrobial susceptibility profile that was representative of the individual organisms on the whole.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Colony Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Enterococci</em></td>
<td>Light Blue</td>
</tr>
<tr>
<td><em>E.coli</em></td>
<td>Pink</td>
</tr>
<tr>
<td>Coliforms</td>
<td>Purple</td>
</tr>
<tr>
<td><em>Proteus/Morganella/Providencia</em> spp</td>
<td>Brown</td>
</tr>
<tr>
<td><em>Pseudomonads</em></td>
<td>Fluoresce</td>
</tr>
<tr>
<td><em>Staphylococci</em></td>
<td>Normal pigmentation</td>
</tr>
</tbody>
</table>
Table 2.3: Antibiogram Interpretation.

<table>
<thead>
<tr>
<th>Penicillin</th>
<th>Tetracycline</th>
<th>Chloramphenicol</th>
<th>Erythromycin</th>
<th>Methicillin</th>
<th>Tetrhemoplin</th>
<th>Gentamycin</th>
<th>Clindamycin</th>
<th>Rifampicin</th>
<th>Vancomycin</th>
<th>Fusidic Acid</th>
<th>Teicoplanin</th>
<th>Ciprofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>Te</td>
<td>C</td>
<td>E</td>
<td>M</td>
<td>W</td>
<td>Cn</td>
<td>Da</td>
<td>Rd</td>
<td>Va</td>
<td>Fd</td>
<td>Tec</td>
<td>Cip</td>
</tr>
<tr>
<td>24</td>
<td>25</td>
<td>19</td>
<td>20</td>
<td>14</td>
<td>15</td>
<td>19</td>
<td>20</td>
<td>19</td>
<td>20</td>
<td>25</td>
<td>26</td>
<td>29</td>
</tr>
<tr>
<td>R ≤</td>
<td>S ≥</td>
<td>R ≤</td>
<td>S ≥</td>
<td>R ≤</td>
<td>S ≥</td>
<td>R ≤</td>
<td>S ≥</td>
<td>R ≤</td>
<td>S ≥</td>
<td>R ≤</td>
<td>S ≥</td>
<td>R ≤</td>
</tr>
</tbody>
</table>

Interpretation of zone diameter (mm)

| 4          | 0            | 2              | 0            | 4           | 0            | 2           | 0          | 1         | 0          | 4            | 0           | 0            |

Discs containing different antibiotics were placed on ISA plates seeded with bacteria. The antibiotics diffuse to create a ZI. If the ZI was greater than the specified diameter (mm) for that antibiotic, the bacteria were classed as being sensitive (S) to that agent. If the ZI was less than the specified diameter for that antibiotic, the bacteria were classed as being resistant (R) to that agent and were given a score. All scores were added up to give an antibiogram number.
2.2.4. The Impregnation Process: Production of Antimicrobial Catheter Material

Rifampicin, sparfloxacin and triclosan were accurately weighed out and added to chloroform to give concentrations (w/v) of 0.2%, 1% and 1% respectively. Once fully solubilised, the silicone material, either the 6 mm silicone discs or the silicone Foley catheters were immersed in the appropriate volume of the antimicrobial solution (50 mL or 250 mL) and left for 1 hour at room temperature. The antimicrobial solution was then removed and the silicone rinsed with ethanol to remove residual chloroform. Samples were left overnight to air dry at room temperature for solvents to evaporate and for the silicone to return to its original size entrapping the antimicrobial agents throughout [154]. Control material was processed in the same manner but without the addition of the antimicrobial agents. All samples were sterilised by autoclaving at 121°C for 15 minutes.

2.2.5. Serial Plate Transfer Test

Uropathogens were sub-cultured onto SBA or CLED to obtain pure cultures. 1 - 3 colonies were added to 5 mL of sterile distilled water to make a 0.5 McFarland suspension. 20 mL ISA plates were prepared and inoculated using a cotton bud stick with bacterial suspension. Antimicrobial impregnated silicone discs were applied to the centre of the plates and incubated overnight at 37°C. Silicone discs without antimicrobial agents served as controls. ZI (minus the disc diameter) were measured using digital callipers and recorded. Those discs showing ZI were removed and transferred to further freshly seeded agar plates, ensuring that the same side of the disc surface was in contact with the agar. All tests were carried out in triplicate. The process was continued until no more ZI were seen, or until the test was terminated at 100 days. If there was still a ZI around the antimicrobial disc but evidence of
bacteria attaching to it, it may be an indication of resistance development. If suspected, the attached bacteria were isolated, their identity compared to the original inoculum to exclude contamination and MIC to the agent/s in the disc determined as shown in Section 2.2.3.

For the SPTT, discs were impregnated with 0.2% rifampicin only, 1% sparfloxacin only, and 1% triclosan only, and in combination (0.2% rifampicin, 1% sparfloxacin, 1% triclosan). The spectrum and duration of activity were established for the single agents and all three agents combined. The other agents listed in Table 2.1 were also screened using the SPTT against several of the uropathogens for their potential duration of activity and resistance development. If the bacteria showed signs of becoming resistant, the MIC of the agent was determined.

2.2.6. Determination of Time Taken to Kill Attached Bacteria

Uropathogens were sub-cultured onto SBA or CLED to obtain pure cultures. A loopful of each was placed into 20mL universal containers containing Tryptone Soya Broth (TSB, Oxoid) and grown to early log phase in a 37°C shaker incubator set at 200 rpm for 4 hours. Universals were centrifuged for 20 minutes at 3000 rpm and re-suspended in a predetermined percentage (%) TSB (refer to section 2.2.6.1). Bacterial suspensions were adjusted to $A_{490} 0.6 - 0.7$ using a Jenway 6705 UV/Vis Spectrophotometer (Fisher Scientific, Leicestershire, UK), which equates to approximately $10^8$ cfu/mL. Suspensions were used at either $10^8$ cfu/mL or diluted in the correct % TSB to approximately $10^5$ cfu/mL.
As highlighted in Section 1.5.3.1 once a urinary catheter has been inserted into the body it rapidly becomes coated in urinary components and a conditioning film forms. A conditioning film was formed on control discs (containing no antimicrobial agents) and antimicrobial silicone discs by exposing them to human urine. The urine (pH 6.8) was sterilised by filtration through a 0.2 µm cellulose nitrate membrane (Whatman, Dassel, Germany) and discs soaked in the urine for 1 hour at 37°C with rocking. Following this discs were removed and placed into 1.5 mL eppendorfs. 1 mL of bacterial suspension was added and incubated for 1 hour at 37°C with rocking to allow for bacterial attachment. The discs were then rinsed in 1 mL of the correct % TSB corresponding to each strain to remove non-adherent bacteria and placed into fresh TSB. Bacteria were detached from the discs by placing the eppendorfs into a sonicator for 20 minutes set at 50 Hz (Ultra Wave Ltd, Cardiff, UK). 200 µL of the detached bacteria were spread onto SBA or CLED plates. Plates were incubated for up to 72 hours and surviving bacteria counted as cfu/mL.

All tests were performed in triplicate and sampled/counted at 0, 24, 48 and 72 hours time points. Each day the discs were rinsed in TSB to prevent antimicrobial build-up and if not tested at that time point were re-incubated. The time taken for the antimicrobial material to kill attached bacteria was determined and compared to control material where attached bacterial counts should remain high.

tK100 tests were also performed on control and antimicrobial silicone discs that had been soaked in phosphate buffered saline (PBS, Oxoid) for 5 days at 37°C. This was to examine the effect that drug elution had on the antimicrobial material’s ability to kill attached bacteria. The PBS was changed daily to prevent the build-up of
antimicrobial agents and to broadly simulate drug elimination within the urinary tract.

2.2.6.1. Percentage TSB Determination

It is important to keep the silicone discs in the correct % TSB, in order to try to supply the attached bacteria with sufficient nutrients to allow them to survive but not for them to vastly increase in numbers [93]. To determine the % TSB to achieve this, control silicone discs were exposed to $10^8$ or $10^5$ cfu/mL of bacteria in varying % TSB concentrations. A tK100 test was performed as in 2.2.6 and the correct % TSB selected according to the concentration at which the attached bacterial counts on the control material remained high and were constant over the 72 hour time period, i.e. counts should remain high and equal to or close to the cfu/mL at 0 hours as at the 72 hour point [93].
2.3. RESULTS

2.3.1. Bacterial Characterisation

Bacteria used throughout the report and their sensitivities to the antimicrobial agents are shown in Table 2.4. They include *S.aureus* (MRSA), *E.coli*, *P.mirabilis*, *K.pneumoniae*, *E.faecalis* and CoNS (*S.saprophyticus*) which represent the organisms most commonly isolated from the urine of patients with CAUTIs and which have a typical antimicrobial sensitivity profile and individual MIC values.
Table 2.4: Identification of bacteria isolated from the urine of patients with a CAUTI.

<table>
<thead>
<tr>
<th>F. No</th>
<th>Organism</th>
<th>API Profile</th>
<th>Antibiogram Profile</th>
<th>MIC (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Rifampicin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Triclosan</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sparfloxacin</td>
</tr>
<tr>
<td>1232</td>
<td><em>S. aureus</em></td>
<td>6736153 97.8% ID</td>
<td>47604</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>MRSA</td>
<td></td>
<td></td>
<td>0.032</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt;3</td>
</tr>
<tr>
<td>1693</td>
<td><em>E. Coli</em></td>
<td>5145552 99.9% ID</td>
<td>46370</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.023</td>
</tr>
<tr>
<td>1691</td>
<td><em>P. mirabilis</em></td>
<td>0437000 95.7% ID</td>
<td>66370</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.094</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.19</td>
</tr>
<tr>
<td>2630</td>
<td><em>K. pneumoniae</em></td>
<td>5215773 97.6% ID</td>
<td>46370</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.032</td>
</tr>
<tr>
<td>2633</td>
<td><em>E. faecalis</em></td>
<td>5143711 99.7% ID</td>
<td>62700</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.38</td>
</tr>
<tr>
<td>2636</td>
<td>CoNS S. saprophyticus</td>
<td>6634152 88.7% ID</td>
<td>46000</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.125</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.19</td>
</tr>
</tbody>
</table>

F. No = Freezer bank identity number. R = Resistant
2.3.2. Serial Plate Transfer Test

Figures 2.5 to 2.8 show the SPTT results of silicone impregnated with rifampicin, sparfloxacin, and triclosan as single agents and in combination (rifampicin, triclosan, sparfloxacin) exposed to MRSA, CoNS, *E. faecalis*, *E. coli*, *K. pneumoniae* and *P. mirabilis*. Control discs showed no intrinsic activity towards the organisms.

2.3.2.1. 0.2% Rifampicin

![Graph showing antibacterial activity](image)

**Figure 2.5:** The antibacterial activity of silicone impregnated with 0.2% rifampicin against MRSA, CoNS, *E. faecalis*, *E. coli*, *K. pneumoniae* and *P. mirabilis* assayed by the SPTT. The assay was performed in triplicate and each point represents the mean with standard error (SE). SE may not be visible at every point as they were very small. *K. pneumoniae* and *E. coli* are superimposed with *P. mirabilis* (displaying no activity towards rifampicin).
MRSA and CoNS were sensitive to rifampicin and *E. faecalis* moderately sensitive, and they displayed a ZI that lasted for 66 days, 24 days and 2 days respectively. On day 24, all three discs exposed to CoNS showed signs of bacteria adhering to the material, indicating resistance. Upon further testing, it was revealed that this was actually cross contamination with *E. coli*, probably due to a failure in aseptic technique whilst transferring the discs of the SPTTs. The test was stopped, but it is likely that unless true resistance developed, the activity would have otherwise continued judging by the ZI diameter compared with MRSA. Figure 2.5 shows large zone diameters, particularly against CoNS and MRSA on Day 1 followed by a rapid decline until a steadier state of release is reached. *E. faecalis* only showed activity lasting for 2 days, possibly relating to the higher MIC of rifampicin. As expected, no zones were displayed against the Gram negative organisms, corresponding to their innate resistance to rifampicin.
2.3.2.2. 1% Sparfloxacin

![Graph showing antibacterial activity of silicone impregnated with 1% sparfloxacin against various bacteria](image)

**Figure 2.6:** The antibacterial activity of silicone impregnated with 1% sparfloxacin against MRSA, CoNS, *E. faecalis*, *E. coli*, *K. pneumoniae* and *P. mirabilis* assayed by the SPTT. The assay was performed in triplicate and each point represents the mean with standard error (SE). SE may not be visible at every point as they were very small.

All bacteria apart from MRSA showed varying degrees of sensitivity to sparfloxacin. *E. coli* was most sensitive and *E. faecalis* the least. By days 6 and 7, sparfloxacin no longer displayed any activity towards *E. faecalis* and *P. mirabilis* respectively. Activity towards CoNS also stopped at day 38, whereas >100 day duration of activity towards *E. coli* and *K. pneumoniae* was shown. For the latter organisms, an initial high release of sparfloxacin is shown followed by a decline until it reached a steady state. Impregnation with sparfloxacin alone showed no signs of resistance.
2.3.2.3. 1% Triclosan

Figure 2.7: The antibacterial activity of silicone impregnated with 1% triclosan against MRSA, CoNS, *E. faecalis*, *E. coli*, *K. pneumoniae* and *P. mirabilis* assayed by the SPTT. The assay was performed in triplicate and each point represents the mean with standard error (SE). SE may not be visible at every point as they were very small.

The concentration of triclosan required to inhibit the growth of *E. faecalis* was the highest at 2 µg/mL compared to the other five bacteria and the shortened duration of activity, <22 days, reflects this. Triclosan showed continued activity towards the remaining bacteria, displaying large zone sizes that continued for 100 days and beyond. Unlike rifampicin and sparfloxacin there appears to be less in terms of an initial burst of the drug but more of a steady persistent release. No sign of resistance was seen over the duration.
2.3.2.4. Combination of Antimicrobial Agents

Figure 2.8: The antibacterial activity of silicone impregnated with a combination of 0.2% rifampicin, 1% triclosan and 1% sparfloxacin against MRSA, CoNS, E.faecalis, E.coli, K.pneumoniae and P.mirabilis assayed by the SPTT. The assay was performed in triplicate and each point represents the mean with standard error (SE). SE may not be visible at every point as they were very small.

All three antimicrobial agents combined showed a 100 day activity towards all of the uropathogens with the exception of E.faecalis. Images of the E.coli SPTT at day 1 and 100 are shown in Figure 2.9 (A and B). The results in Figure 2.8 are similar to those with material impregnated with triclosan alone. Results generated from the SPTT performed with silicone discs impregnated with single antimicrobial agents provided information on the duration of activity to which bacteria are sensitive to one or more of the drugs. E.coli and K.pneumoniae were affected by triclosan and sparfloxacin over the 100 day duration and CoNS probably by rifampicin and
triclosan over a prolonged period with sparfloxacin giving short-term activity. *P. mirabilis* was affected mostly by triclosan alone with sparfloxacin activity in the short-term and MRSA by rifampicin and triclosan. *E. faecalis* showed only a short-term duration of <22 days using this combination with activity coming from triclosan. No signs of resistance were seen.

### 2.3.2.5. Serial Plate Transfer Test and Depiction of Resistance

In selecting suitable antimicrobial agents from Table 2.1, all were screened against several of the microorganisms using the SPTT (not all data shown). Impregnation of the silicone discs with trimethoprim alone repeatedly exposed to *K. pneumoniae* showed signs of resistance on day 38 of transfer as shown in Figure 2.9 (C). Upon identification and MIC testing, the bacteria from day 38 were found to be resistant to trimethoprim (>32 µg/mL compared to the original MIC of 0.38 µg/mL). No bacterial resistance developed when bacteria were exposed to the other agents listed in Table 2.1, either as single agents or when combined.
Figure 2.9: SPTT of rifampicin, sparflaxacin, triclosan (combination) impregnated silicone discs exposed to *E.coli* on (A) Day 1 and (B) Day 100 and (C) *K.pneumoniae* exposed to material containing 1% trimethoprim, showing signs of resistance. Though there is a ZI, the bacteria that are attached to the disc are surviving and growing on it.
2.3.3. Time Taken to Kill 100% of Attached Bacteria

Figures 2.10 and 2.11 show the results of the time taken for the rifampicin, triclosan and sparflxacin containing silicone to kill ideally 100% of attached bacteria. The correct % TSB to perform the assay with was established and is shown in Table 2.5. A urinary conditioning film was formed on control and antimicrobial silicone discs, pre and post soaking/drug elution, and prior to being exposed to $10^8$ cfu/mL or $10^5$ cfu/mL of *E.faecalis*, CoNS, MRSA, *E.coli*, *K.pneumoniae* or *P.mirabilis*.

Table 2.5: Determination of percentage TSB required to maintain bacterial viability.

<table>
<thead>
<tr>
<th>Organism</th>
<th>TSB (%) upon exposure to $10^8$ cfu/mL bacteria</th>
<th>TSB (%) upon exposure to $10^5$ cfu/mL bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>CoNS</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>E.faecalis</em></td>
<td>0.1</td>
<td>1</td>
</tr>
<tr>
<td><em>E.coli</em></td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td><em>K.pneumoniae</em></td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td><em>P.mirabilis</em></td>
<td>0.5</td>
<td>1</td>
</tr>
</tbody>
</table>
Figure 2.10: tK100 results for material impregnated with 0.2% rifampicin, 1% triclosan, 1% sparﬂoxacin and controls. Material was exposed to urine for 1 hour to form a conditioning ﬁlm prior to exposure to (A) 10^8 cfu/mL E. faecalis, CoNS, MRSA, and (B) E.coli, K.pneumoniae at 10^8 cfu/mL and P. mirabilis at 10^5 cfu/mL respectively. After, material was sonicated at time intervals and the viable bacteria in the sonicate counted. Each point represents the mean of three tests with standard errors. Figure A shows lines for CoNS and MRSA which are superimposed.
The tK100 results for discs impregnated with rifampicin, triclosan and sparfloxacin with a conditioning film show that no viable CoNS, MRSA or *K.pneumoniae* cells remained attached by the end of 24 hours, or *E.coli* by 48 hours and *E.faecalis* by 72 hours. Material exposed to $10^8$ cfu/mL *P.mirabilis* (data not shown) was not able to kill all of the attached bacteria. Upon exposure to $10^5$ cfu/mL, <25 cfu/mL remained attached at 72 hours. Bacterial numbers attached to control discs remained steady throughout at approximately $10^5$ cfu/mL.
**Figure 2.11:** tK100 results for material impregnated with 0.2% rifampicin, 1% triclosan, 1% sparfloxacin and controls. The material was soaked in PBS for 5 days to simulate drug release followed by exposure to urine for 1 hour to form a conditioning film prior to exposure to (A) $10^5$ cfu/mL *E. faecalis*, CoNS, and MRSA, and (B) $10^5$ cfu/mL *E. coli*, *K. pneumoniae* and *P. mirabilis* respectively. After, material was sonicated at time intervals and the viable bacteria in the sonicate counted. Each point represents the mean of three tests with standard errors.
Following soaking to simulate drug release, the rifampicin, triclosan, sparfloxacin combination reduced the number of bacteria attached to the materials surface but did not eradicate all of them when exposed to a $10^8$ cfu/mL inoculum (data not shown). A lower inoculum of $10^5$ cfu/mL was therefore used. Although small numbers of bacteria adhered to the antimicrobial discs when exposed to $10^5$ cfu/mL bacteria, the trend for bacteria adhering to control discs was to increase over time where the opposite effect was shown for the antimicrobial discs. *E.coli* and *K.pneumoniae* were eliminated within 24 hours, *E.faecalis* within 48 hours and CoNS and MRSA within 72 hours. $>50$ cfu/mL *P.mirabilis* remained attached to the antimicrobial discs.
2.4. DISCUSSION

The SPTT is based on the ability of the antimicrobial agents to diffuse out of the polymer to create a ZI against susceptible organisms. It is a static test that can be used to screen the antimicrobial agents potential duration of activity against target organisms due to its serial nature. A variety of antimicrobial agents shown in Table 2.1 were investigated for impregnating into silicone discs and the spectrum and duration of activity against a range of CAUTI organisms assessed using the SPTT as an initial screening test.

Having successfully been incorporated into bone cements as a means of applying localised antibiotic delivery [155], gentamicin sulphate was investigated but is insoluble in chloroform so was ruled out. Similarly minocycline having been used as coatings on various catheters to prevent microbial colonisation was not suited to the solvent - based impregnation process as it is also poorly soluble in chloroform. On screening clindamycin and rifampicin it appeared that long lasting activity in particular against staphylococci was possible from using a 0.2% concentration in the chloroform [81].

Yassien et al (1995) [156] studied the effects of four types of quinolones on reducing biofilms formed on vascular catheters in an in - vitro model. They found that a concentration of agents 4 - 25 times the MIC of planktonic P.aeruginosa showed <5% growth of adherent cells compared to the controls. The effect of quinolones on actual catheter biofilms and reports of incorporation into material to prevent progression to the biofilm state [118] led to viewing quinolones for potential activity
and duration against uropathogens. Sparfloxacin and norfloxacin were readily soluble in chloroform based on a 1% concentration [157], and gave a broad spectrum of activity and the potential of a long duration upon screening. They were also investigated as some microorganisms that are resistant to fluoroquinolones may be sensitive to the lesser-used agents. Sparfloxacin was found to give longer lasting activity against Gram negative bacteria compared to norfloxacin (data not shown).

Incorporating trimethoprim into polymers along with rifampicin in an in-vitro model showed that S.aureus colonisation was reduced by >99.97% [158]. Similarly trimethoprim, rifampicin and triclosan incorporated into peritoneal dialysis catheters has shown to inhibit bacterial colonisation by E.coli and MRSA in an in-vitro model following repeated bacterial challenges for 90 days [157]. Upon screening, trimethoprim (1%) showed a prolonged duration of activity particularly against Gram negative organisms (data not shown) but resistance was generated to K.pneumoniae (Figure 2.9). This agrees with research [159] which has highlighted that as a drug commonly used to treat UTIs, resistance to trimethoprim is increasing and this could be an issue if many of the organisms isolated from patients with CAUTIs have been treated with trimethoprim in the past.

The potential of antiseptics were also investigated. Hexetidine and octenidine dihydrochloride are said to have a broad spectrum of activity but when incorporated into silicone even at concentrations of 5%, they displayed only a small ZI against common uropathogens which was short lived (data not shown). There is much scientific research as highlighted in section 1.6.5 which indicates that triclosan is highly effective in reducing bacterial colonisation. The SPTT of triclosan
impregnated silicone discs using a 1% concentration in the solvent [157] displayed a broad spectrum of activity with zone diameters that looked likely to continue for a long duration. Silver was not assessed in this project.

Of the antimicrobial agents screened, clindamycin or rifampicin could act to affect predominantly Gram positive organisms especially the staphylococci. Sparfloxacin or trimethoprim could act to affect mostly Gram negative bacteria but give some Gram positive protection whilst triclosan could cover both. Clindamycin and sparfloxacin however upon combination did not combine well and analytical grade clindamycin powder is an expensive drug to use for testing purposes. Due to the increasing reports of resistance to trimethoprim, a combination of 0.2% rifampicin, 1% triclosan and 1% sparfloxacin was therefore selected to become impregnated into the urinary catheter.

Several studies [160, 161] have compared the efficacies of antimicrobial catheters using the ZI test but zone diameters were only measured after overnight incubation and no further tests performed. By transferring the material to freshly seeded agar plates we can see that often there is a sudden drop in antimicrobial activity after the first day. For example, Figure 2.5 shows that *E. faecalis* when exposed to silicone containing rifampicin, the initial zone diameter was ~18 mm but by the third day no zone of inhibition remained. This indicates that in some instances activity may be limited to a few days and is an effect of surface activity and it is important to see if the activity is likely to continue thereafter rather than draw conclusions from a one-day zone of inhibition test [157].
The SPTT showed that rifampicin incorporated as a single agent into the silicone discs gave a duration of activity that persisted for 65 days against MRSA and despite the contamination of all three discs exposed to CoNS, it appeared from the zone sizes that the duration of activity against CoNS would have otherwise continued. To verify this, the experiment should have been repeated. Sparfloxacin was active against *E.coli* and *K.pneumoniae* over the whole 100 day SPTT duration and displayed some shorter term Gram positive (CoNS) activity. Triclosan showed a broad spectrum of prolonged activity with the exception of *E.faecalis* (22 days). *E.faecalis* is an organism commonly isolated from the urine of patients on LTC and the short duration of antimicrobial activity against it may be of concern and could be related to the higher MIC values. However, the impact that *E.facealis* has on symptomatic CAUTI requires further investigation. Combining all three agents displayed persisting activity against the uropathogens which gradually declined over the 100 days, with the exception of *E.faecalis*. It is anticipated that if the bacteria are sensitive to the individual antimicrobial agents and display a persisting ZI, the agents combined should act in a manner similar to the agents by themselves but synergistically to provide protection from the emergence of resistance. Protection provided by two antimicrobial agents (sparfloxacin and triclosan) looked possible for *E.coli* and *K.pneumoniae* over the whole SPTT duration and for a long duration towards CoNS and MRSA (rifampicin and triclosan). *P.mirabilis* was mostly acted upon by triclosan alone over the 100 day duration and *E.faecalis* by triclosan but only short-term.

The SPTT can also be used to detect the development of resistance which can be visualised by microbial growth around or under the impregnated silicone material. It
emerged that *K. pneumoniae* became resistant at day 38 following repeat exposure to material impregnated with trimethoprim alone, with the MIC increasing to >32 µg/mL. It is proposed that as only one side of the material is exposed to the bacteria, if the level of trimethoprim reaching the surface was approximating the MIC of the organism or was sub - inhibitory over a prolonged period then it is possible that the bacteria would mutate to a more resistant phenotype which will allow survivors to remain attached to the disc and a rise in MIC occur [157]. The Dual Drug Principle [122] works on the basis that if bacteria are to survive they must undergo simultaneous resistance mutations in two different genes distant in the genome and the chances of this are extremely low if more than one antimicrobial agent is used at correct concentrations. By applying this principle thus far, the SPTT has not shown bacteria to develop resistance towards the rifampicin, sparfloxacin, triclosan combination.

Studies by Sherertz *et al* (1993) [161, 162] suggested that there was a relationship between *in - vitro* anti-infective catheter zones of inhibition and *in - vivo* efficacy. They report that a >15 mm ZI against *S. aureus* was necessary to prevent bacterial colonisation in a rabbit model and that the concept may be generalizable to other organisms. A study by Tambe *et al* (2001) [163], however, found that catheters containing chlorhexidine, chlorhexidine acetate and silver sulphadiazine formed small sized zones after soaking (<7.2 mm) upon exposure to *S. epidermidis* but were able to kill the adhered bacteria. On the other hand the minocycline and rifampicin catheters showing larger zone sizes (14 mm) were unable to prevent colonisation by *S. epidermidis*. They suggest that zone size may be related to the solubility and diffusion characteristics of the drugs, while the ability to resist bacterial colonisation
may depend on the type of antimicrobial action (static or cidal) and slow and rapid kill [163].

The SPTT is a useful screening tool but in this study was not used to assess the ability of the antimicrobial combination to provide protective activity against bacterial colonisation. The antimicrobial catheter works by preventing bacterial colonisation rather than by the inhibition of adherence [152] and the tK100 assay addresses this by attaching the bacteria to the antimicrobial material and monitoring the time it takes for the agents to kill the attached bacteria. Due to the length of time the tK100 assay is run (72 hours) it is important to establish the appropriate TSB concentration which is used to support the bacteria. The correct TSB concentration should ensure bacterial viability but not allow for too much multiplication and this was determined for each bacterium as shown in Table 2.5. Too high a % TSB can cause exponential growth whereas too low a % TSB can result in insufficient nutrients being available causing the bacteria to die off.

Initially to ensure that bacteria became attached to the material, it was exposed to a $10^8$ cfu/mL suspension of bacteria. Some bacterial species (\textit{P.mirabilis}) remained attached to the material after the 72 hour test period and all species remained attached (data not shown) following soaking of the material when exposed to $10^8$ cfu/mL. Following this, it was thought that $10^8$ cfu/mL was too high a number to be exposing the material to, so for some assays challenge with $10^5$ cfu/mL bacteria was adopted. This was deemed to be a reasonable level of bacteria to expose the silicone to as in clinical practice a CAUTI is diagnosed if $>10^3$ cfu/mL of a predominant bacteria is isolated [32, 33]. The numbers of bacteria attached to the control discs
when exposed to $10^8$ cfu/mL remained relatively constant over the 72 hour test period whereas the numbers attached to the control discs following exposure to $10^5$ cfu/mL were low at 0 hours and increased with time. Bacterial numbers attached to the antimicrobial material showed the opposite effect and decreased over time, revealing the ability of the agents to release from the matrix and kill the number of attached bacteria.

It is also important during the tK100 assay to take into account the effect conditioning films have on bacterial colonisation. Conditioning films were formed on the silicone discs by exposing them to human urine for 1 hour. X-Ray Photoelectron Spectroscopy (XPS) was used to confirm that 1 hour exposure to urine was sufficient time for a conditioning film layer to be formed on the catheter surface and this is discussed further in Chapter 5 (Surface Analysis). The antimicrobial catheter was found to be capable of killing attached bacteria in the presence of a urinary conditioning film. There has, however, been conflicting reports concerning the effect that conditioning films have on bacterial attachment. A study by Gorman et al (1997) [49] examined the adherence of *S.epidermidis* to silicone peritoneal catheters in the presence and absence of a conditioning film formed by exposing the material to artificial spent peritoneal dialysate (ASD) for 1 hour. They found that treatment with ASD decreased the adherence of *S.epidermidis*. Murga et al (2001) [164] investigated the effect that a conditioning film formed by exposing central venous catheter connections to blood had on biofilm formation caused by Gram negative bacteria. They reported that the conditioning film formed of blood components promoted biofilm formation. In urine, 67 protein forms from which 47 unique proteins have been isolated and identified [165]. Santin et al (1999) [166] in
an *in-vitro* model demonstrated that a conditioning film formed by exposure to urine enhanced crystal precipitation on the catheter surface but they did not study bacterial colonisation per se. The studies highlight the differences in results and the importance of taking into account the effect conditioning films may have on bacterial colonisation.

Kohnen et al (2003) [118] report on silicone ventricular catheters impregnated with rifampicin and sparfloxacin challenged with $10^7$ cfu/mL of *S.epidermidis* in a time kill assay. They found a 99.8% reduction in attached bacteria compared to catheter segments with no antimicrobial agents. Following soaking of the material for up to 1 year there was still a 99.8% reduction in bacterial colonisation compared to control ventricular catheters. Tambe et al (2001) [163], however, showed that the mean adherence of *S.epidermidis* isolates to minocycline and rifampicin catheters increased following up to 24 days soaking. The rifampicin, sparfloxacin, triclosan containing silicone discs showed a reduction in ability to kill attached bacteria following 5 days soaking. As the agents eluted, the material was unable to kill all attached bacteria that were applied as a $10^8$ cfu/mL initial load (data not shown). It was, however, more capable of killing attached bacteria that were applied as a $10^5$ cfu/mL initial load and eliminated all attached bacteria within a 72 hour time period with the exception of 50 cfu/mL *P.mirabilis* remaining.

*P.mirabilis* was not completely eradicated by the action of the antimicrobial agents before or after the 5 day drug elution. The effect of a few colonies remaining attached to the material in its ability to prevent bacterial colonisation will need to be examined further in a flow model system. Even though *P.mirabilis* remained after
72 hours, compared to the control material a >99.9% reduction of attached bacteria was shown. A tK100 was, however, achieved for both soaked and un-soaked material following challenge with CoNS, MRSA, *E. faecalis*, *E.coli*, and *K.pneumoniae* within 24 to 72 hours. Despite a short duration of activity displayed by the SPTT towards *E.faecalis*, the tK100 showed the agents to be effective in killing attached *E.faecalis*. This is likely to be due to the action of triclosan over the 72 hours but is not to say that the antimicrobial material would be effective if challenged further with *E.faecalis*.

It can be seen from the tK100 test that the antimicrobial material did prevent the colonisation of the majority of the uropathogens tested. Although this is a static test and applies only one bacterial challenge to the material, the duration of activity shown by the SPTT reveals that there is potential that the material may be successful in preventing colonisation if challenged further with certain organisms. The burst effect from sparfloxacin and rifampicin and the sustained release of triclosan shown from the SPTT along with the tK100 results have demonstrated that the antimicrobial combination may be effective in preventing bacterial colonisation at the time of catheter insertion, in particular against CoNS, MRSA, *E.coli* and *K.pneumoniae*. Urinary catheters are, however, exposed to potential bacterial entry at any point during their use, so a long-lasting effect is required if infection is to be reduced. An *in-vitro* model simulating flow conditions within the bladder that subjects the material to repeated bacterial challenges will help to determine the effectiveness of the antimicrobial catheter in preventing bacterial colonisation over a longer term.
CHAPTER 3

IN - VITRO MODEL
3.0. **In - Vitro Model**

3.1. **INTRODUCTION**

Despite the problems caused by bacterial colonisation on urinary catheters there is no standard *in - vitro* test to quantify this. There are, however, several types of models reported in the literature that examine ways of studying the effectiveness of drug releasing catheters at inhibiting bacterial colonisation and encrustation and are discussed in section 3.1.2. Ideally controlled human clinical trials are the best way of assessing the effectiveness of medical devices but these can be slow to set up, expensive and often evaluate small numbers of patients [167]. The variability of human models in terms of diet, fluid intake, urinary constituents and infection by a wide range of organisms has made comparing biomaterials difficult because any real differences in the biomaterials are confounded by other uncontrollable variables [168], however, in order for a medical device to gain CE marking and MHRA (Medicines and Healthcare Products Regulatory Agency) clearance, human studies are essential. To assess safety, tolerability, pharmacokinetics and pharmacodynamics of a product, Phase I clinical trials involving a small number of humans is required. Progression to Phase II trials involving larger numbers of people to assess how well the product works and Phase III involving a randomised controlled multicentre trial to assess how effective the product is compared to the current ‘gold standard’ are then required to establish suitability for marketing. To progress to human studies, pre - clinical (*in - vivo* and *in - vitro*) studies are required in the first instance to demonstrate safety of the device and to confirm proof of principle. Animal models can be more easily controlled than humans in terms of
fluid and diet but their urinary constituents can still vary and differences in their pharmacodynamic ability compared to humans can make efficacy evaluation problematic. To gain knowledge into how a medical device may perform in the body, it is common for *in vivo* studies to be supplemented by *in vitro* studies.

### 3.1.1. The Ideal *In Vitro* Model

In an ideal *in vitro* model of medical device efficacy, all variables should be controlled except the biomaterial under review, thus allowing in this instance to determine the direct effect the material has in preventing bacterial colonisation.

Choong *et al* (2000) [168] stated that the ideal model should include: control of pH and temperature, uniform exposure of bacteria to the material, have a flow of urine mimicking human urinary flow rates which should flow evenly over the material and not be recycled. Separate chambers for test and control material to prevent cross-contamination should be provided, results must be reproducible and the model should be simple, of low cost and be able to be adapted to accommodate a variety of catheter sizes. There are of course many differences between *in vitro* and *in vivo* models that cannot be accounted for in laboratory models. The lack of host defences such as white blood cells, immunoglobulins, complement and cytokines, fever in patients, the diversity of bacteria, microbial load and the virulence of organisms are a few examples [167]. The complexities of infection are difficult to simulate in an *in vitro* model but they can give pointers to the extent an antimicrobial catheter may affect bacterial colonisation in order to satisfy regulatory requirements for subsequent testing in humans.
3.1.2. *In Vitro* Models Used to Study the Effectiveness of Drug-Containing Materials

Of the few catheterised urinary models cited [7, 168, 169], many report on encrustation of urethral catheters. The better-designed models take the form of two-compartmental type models. They generally consist of a vessel connected to input and output ports through which medium can flow. This is known as the central compartment and represents the blood and equilibrating tissue sites [167]. Inserted into the central compartment is a peripheral compartment which corresponds to the tissue site containing the infection [167]. Two-compartment models can be modified and made more specific to what is being investigated. An example of a glass bladder model used by Stickler *et al* (1999) [7] and Barford *et al* (2008) [169] to investigate the impact that different urinary catheters have on encrustation by *P.mirabilis* is depicted in Figure 3.1.
Figure 3.1: Model of the catheterised urinary tract detailing one of the bladders with a catheter in situ [7, 169].
The glass vessel represents the bladder through which a urinary catheter tip is inserted that extends through a glass tube representing the urethra. Urine is pumped into the glass bladder which flows through the catheter to a drainage bag. Bacteria are inoculated directly into the urine within the glass bladder and as the bacterially infected urine flows through the catheter lumen, encrustation and biofilm formation can be studied. Human urine, artificial urine or laboratory culture medium can be used. Human urine is very variable and if large volumes are required over a prolonged period, ensuring a constant supply may be problematic. Artificial urine has the advantage that its composition can be controlled but there are many constituents in human urine that can inhibit or promote bacterial colonisation/encrustation which are not present in artificial urine. Filtration of the quantities of artificial urine required may also be difficult to keep supplied over a long course. As urinary fluid is a complex milieu whose contents can influence bacterial adhesion to substrata [170], models often use laboratory nutrient broths to give an indication of the likely effect of the test material under review.

In a study by Choong et al (2000) [168], a model of encrustation similar to that shown in Figure 3.1 was devised for the purpose of validating in - vitro performance with in - vivo results. Catheter material was subject to pooled human urine at a flow rate of 0.5 mL/min over a course of 5 days. To ensure that bacteria did not grow in the urine, gentamicin and vancomycin were added. They reported that in - vitro results of the effectiveness of biomaterials in preventing encrustation correlated well with in - vivo implantation into the bladder of rats.
Tunney et al (1999) [171] designed a continuous flow model to study biofilm development and encrustation by *P.mirabilis* on urethral catheters. It was based on a modified Robbins device where artificial urine containing the bacteria was re-circulated at 0.5 mL/min through tubing feeding to pieces of test catheter. Intraluminal encrustation was formed and studied. In investigating the prevention of bacterial colonisation of urinary catheters, urine should ideally not be re-circulated and to add antimicrobial agents to urine to prevent bacterial growth as in the Choong et al (2000) [168] study may hide away the effect the drug-releasing catheter has on preventing bacterial colonisation.

An example of a further model is the catheter bridge model reviewed by Kazmierska et al (2010) [172] which studies the migration of *P.mirabilis* over catheter sections as shown in Figure 3.2. It uses an agar plate through which a horizontal channel is cut where the catheter segment lies. Bacteria are inoculated one side of the channel and migrate over catheter segments. In this instance material coated in a hydrogel with and without iodine was studied to see how effective the material was at preventing the bacteria from reaching the other side of the catheter.
Figure 3.2: Catheter bridge model depicting the migration of *P. mirabilis* over catheter segments coated with hydrogel and hydrogel plus antibacterial agent.

*P. mirabilis* was found to move quicker through the hydrogel-coated catheter and slower when it was combined with iodine. The model however is a static test and is not useful for studying the potential duration of activity that a drug-releasing material has and is suitable only for organisms that are motile. An improved version of this could involve the migration of *Proteus* inside the test catheter from one end to the other under flow conditions using a pump system. In general, the study of catheter colonisation in the absence of confounding biological factors could be modelled as a catheter with fluid flow and bacteria attached to the inner surface. To examine the potential duration of protective activity an antimicrobial catheter gives, the catheter could be repeatedly challenged with bacterial loads.
3.1.3. Objectives

The aim of this chapter is to develop a novel *in-vitro* model that represents the catheterised urinary tract and to evaluate the ability of the antimicrobial catheter to resist bacterial colonisation. It is anticipated that the antimicrobial catheter under flow conditions would be able to prevent bacterial colonisation and the development of resistance over a prolonged period following repeat weekly challenges with high doses of bacteria. The *in-vitro* model along with drug content and release analysis, could be used to determine if the dosing regimen is sufficient to maximise the eradication of the pathogens and if it is effective at limiting the development of resistance [167].
3.2. METHODS

3.2.1. Growth Curve to Assess Bacterial Growth Rates in TSB and Artificial Urine

Ideally in - vitro models designed to test bacterial colonisation on urinary catheters require real or artificial urine as the perfusion medium. A flow rate of 0.5 mL/min would require each catheter to be perfused with 720 mL/day. With each antimicrobial catheter being tested in triplicate plus controls and 2 or 3 bacterial species being tested in any 12 week testing period this amounts to between 8 to 12 L of urine required each day. Artificial urine described by Griffith et al (1976) [173] and as shown in Section 3.2.1.1 was examined for use in the in - vitro model. Difficulties in the ability to sterilise the quantity of artificial urine via membrane filtration and the time it took led to the search for an alternative medium that would support bacterial growth characteristics similar to those in urine. Artificial urine could also not be sterilised by autoclaving. Bacterial growth rates in pure TSB were therefore compared to those in artificial urine over a period of 8 hours.
Chapter 3 - *In Vitro* Model

1. One isolated bacterial colony from a SBA or CLED plate was removed using a steril loop, inoculated into 10 mL sterile TSB and incubated overnight at 37°C.

2. 0.5 mL was inoculated into 50 mL TSB and 50 mL artificial urine (Table 3.1).

3. The cultures were placed into a 37°C orbital incubator set at 200 rpm.

4. Every hour for 8 hours, 100 µL of the bacterial suspensions were removed for bacterial quantification. Ten-fold dilutions were made as necessary and 200 µL of the appropriate dilution plated out and spread evenly onto SBA or CLED.

5. Plates were incubated overnight at 37°C and cfu/mL recorded.

6. Graphs of time against log bacterial counts/mL were plotted.

### 3.2.1.1. Artificial Urine

**Table 3.1:** Recipe for artificial urine [173].

<table>
<thead>
<tr>
<th>Amount: g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium Chloride</td>
</tr>
<tr>
<td>Calcium Chloride</td>
</tr>
<tr>
<td>Potassium Chloride</td>
</tr>
<tr>
<td>Sodium Chloride</td>
</tr>
<tr>
<td>Magnesium Chloride Hexahydrate</td>
</tr>
<tr>
<td>Sodium Sulphate</td>
</tr>
<tr>
<td>Trisodium Citrate Dihydrate</td>
</tr>
<tr>
<td>Disodium Oxalate</td>
</tr>
<tr>
<td>Potassium Dihydrogen Phosphate</td>
</tr>
<tr>
<td>Gelatin</td>
</tr>
<tr>
<td>Urea</td>
</tr>
</tbody>
</table>

The above chemicals (Sigma-Aldrich) were accurately weighed out and dissolved in 1 L sterile distilled water. The pH was adjusted to 6.1 and sterilisation by membrane filtration through a 0.2 µm pore using a vacuum system was employed. TSB was sterilised separately by autoclaving and added to the above to give a final concentration of 10 g/L.
3.2.2. **Principle of the In - Vitro Model**

All silicone Foley catheters were used in either their “as received” state for control purposes or impregnated with rifampicin, triclosan and sparfloxacin as described in Section 2.2.4. Section 3.2.2.1 to 3.2.2.3 detail the *in - vitro* model set, assembly, testing and sampling procedure and Figure 3.3 depicts this. TSB was used as the perfusion medium at a flow rate of 0.5 mL/min. The lumens of the urinary catheters were inoculated with $10^5$ cfu/mL of bacteria suspended in TSB (*E.coli, E.faecalis, K.pneumoniae, MRSA, P.mirabilis*) and catheter ends clamped for 1 hour to allow the bacteria in the suspension to attach to the luminal surface. The tK100 screening tests and previous studies have indicated that 1 hour exposure time is sufficient for bacteria to become attached to material [81, 174]. The flow was restarted and samples of perfused TSB taken from the distal end of the catheters were collected on a daily/near to daily basis. 200 µL was plated out onto SBA or CLED and incubated for up to 72 hours. Surviving colonies were counted. If no bacterial counts were detected, catheters were re - challenged on a weekly basis and new control catheters set up. Water surrounding the catheter in the glass tube was replaced on a weekly basis to prevent the build - up of antimicrobial agents. Tests were carried out at 37°C in triplicate. Challenges were made for up to 12 weeks or until the antimicrobial catheters failed to stop bacterial colonisation. Bacteria isolated from colonised antimicrobial catheters were identified and the MIC of rifampicin, sparfloxacin and triclosan determined as detailed in Section 2.2.3 and Appendix 1. All test catheters and a colonised control catheter from each of the bacterial species were subject to evaluation by Scanning Electron Microscopy (SEM).
Figure 3.3: In-vitro model.
3.2.2.1. Description of In - Vitro Model Set - Up

**Part 1: Perfusion Fluid Reservoir**

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2 L flasks of TSB were made up as per manufacturer’s instructions</td>
</tr>
<tr>
<td>2</td>
<td>A 3 mm hole was made through the bung/lid of the flask and 1 x 3 mm tubing (SLS, Nottingham, UK) thread through until it reached the bottom of the fluid</td>
</tr>
<tr>
<td>3</td>
<td>A glass connector (1 mm diameter) was inserted into the other end of the 1 x 3 mm tubing</td>
</tr>
<tr>
<td>4</td>
<td>A clamp was placed onto the tubing to ensure the fluid does not escape</td>
</tr>
<tr>
<td>5</td>
<td>The top of the flask and the end of the tubing was covered with aluminium foil and secured with tape</td>
</tr>
<tr>
<td>6</td>
<td>All was sterilised by autoclaving. TSB was renewed as necessary</td>
</tr>
</tbody>
</table>

**Part 2: Inlet Tubing**

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A glass connector was pushed into one end of the pump tubing</td>
</tr>
<tr>
<td>2</td>
<td>Attached to the above glass connector was 1 x 3 mm tubing</td>
</tr>
<tr>
<td>3</td>
<td>A short piece of 2 x 4 mm tubing (SLS, Nottingham, UK) was attached to the other end of the 1 x 3 mm tubing</td>
</tr>
<tr>
<td>4</td>
<td>A straight connector 3 - 5 mm (SLS, Nottingham, UK) was connected to the 2 x 4 mm tubing</td>
</tr>
<tr>
<td>5</td>
<td>The end of the connectors were covered with aluminium foil and sterilised by autoclaving</td>
</tr>
</tbody>
</table>

**Part 3: Catheter Section**

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A suitable sized hole was made through the 14 x 20 mm silicone stoppers (SLS, Nottingham, UK) that fit into the glass tubes within the water jacket</td>
</tr>
<tr>
<td>2</td>
<td>The control and antimicrobial catheters were cut to size (minus both ends of the catheter) and pushed through the hole in the silicone stopper until approx 2.5 cm poked through each end</td>
</tr>
<tr>
<td>3</td>
<td>Ends were covered with aluminium foil and sterilised by autoclaving</td>
</tr>
</tbody>
</table>
### Part 4: Outlet Tubing

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>To one end of a straight connector a small section of 2 x 4 mm tubing was attached</td>
</tr>
<tr>
<td>2</td>
<td>1 x 3 mm tubing was inserted into the end of the 2 x 4 mm tubing</td>
</tr>
<tr>
<td>3</td>
<td>Ends of the tubing and connector were covered with aluminium foil and sterilised by autoclaving</td>
</tr>
</tbody>
</table>

### Part 5: Waste Collection Vessel

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A 30 L Sharps Container was used for the collection of waste perfusion medium (TSB)</td>
</tr>
<tr>
<td>2</td>
<td>Disinfectant (Trigene) was added to kill bacteria within waste perfusion fluid</td>
</tr>
</tbody>
</table>
### 3.2.2.2. Assembly of *In Vitro* Model

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The water jacket was filled with distilled water and maintained at 37°C by the heating circuit</td>
</tr>
<tr>
<td>2</td>
<td>From Part 2 of the inlet section, the pump tubing was clipped into place on the pump</td>
</tr>
<tr>
<td>3</td>
<td>The 1 x 3 mm tubing from Part 1 of the perfusion reservoir was connected to the glass connector of the pump tubing</td>
</tr>
<tr>
<td>4</td>
<td>The catheter from Part 3 was inserted up the centre of the tubes in the water jacket and the silicone stopper pushed securely into the bottom of the glass tubes</td>
</tr>
<tr>
<td>5</td>
<td>The glass tubes of the water jacket were filled with distilled water to 2 cm from the top and Parafilm wrapped around the top where the catheter protruded</td>
</tr>
<tr>
<td>6</td>
<td>The straight connector from Part 2 of the inlet tubing was fitted into the top of the catheter</td>
</tr>
<tr>
<td>7</td>
<td>To the bottom of the catheter, the straight connector from Part 4 of the outlet tubing was pushed through</td>
</tr>
<tr>
<td>8</td>
<td>The end of the 1 x 3 mm tubing from Part 4 of the outlet tubing was inserted into the waste collection vessel. The tubing was held in place with tape to ensure that it did not touch the sides of the container. For each bacterial strain separate waste containers for both the antimicrobial and control catheters were used</td>
</tr>
<tr>
<td>9</td>
<td>The clamp from the tubing attached the perfusion reservoir in Part 1 was removed and the pump started at a flow rate of 0.5 mL/min. Checks were made to ensure that no leaks occurred and that the TSB flowed through unimpeded</td>
</tr>
<tr>
<td>10</td>
<td>The system was allowed 1 hour to stabilize and reach 37°C before the catheters were challenged with bacteria</td>
</tr>
</tbody>
</table>
3.2.2.3. Testing and Sampling Procedure

Several colonies of MRSA, *E.coli*, *E.faecalis*, *K.pneumoniae*, or *P.mirabilis* from overnight SBA or CLED plates were placed into separate universal containers of 20 mL of TSB. These were incubated in a 37°C orbital shaker set at 200 rpm for 4 hours and grown to early log phase. Cultures were standardised to achieve an optical density (490 nm) of between 0.6 - 0.7 using a Jenway 6705 UV/VIS spectrophotometer. This gives approximately $10^8$ cfu/mL which was diluted in TSB to $10^5$ cfu/mL and inoculated down the lumens of the catheters.

### Inoculation Procedure

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A clamp was placed onto the inlet tubing and the pump turned off</td>
</tr>
<tr>
<td>2</td>
<td>The straight connector on the top of the catheter was removed and the tip of the catheter swabbed using a steret</td>
</tr>
<tr>
<td>3</td>
<td>A 2 mL syringe was filled with the bacterial suspension and injected down the catheter lumen trying to avoid any air bubbles</td>
</tr>
<tr>
<td>4</td>
<td>Once the bacterial suspension reached the distal end of the catheter, the outlet tubing was clamped</td>
</tr>
<tr>
<td>5</td>
<td>The area around the inoculation site was swabbed with a steret and the connector replaced</td>
</tr>
<tr>
<td>6</td>
<td>The system was left for 1 hour to allow the bacteria to attach to the catheter surface</td>
</tr>
<tr>
<td>7</td>
<td>After 1 hour, the clamp attached to the outlet tubing was removed followed by the clamp attached to the inlet tubing</td>
</tr>
<tr>
<td>8</td>
<td>The pump was restarted and the perfusion medium flowed through for approximately 1 hour</td>
</tr>
<tr>
<td>9</td>
<td>The inlet tubing with connector section and the outlet tubing with connector section were then renewed to prevent the migration of bacteria that may have attached to the silicone tubing from travelling to the test catheter</td>
</tr>
</tbody>
</table>
### Sampling Procedure

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The outlet tubing (Part 4) connected from the distal end of the urinary catheter was disconnected for sampling</td>
</tr>
<tr>
<td>2</td>
<td>A 7 mL bijou bottle was placed under the end of the catheter and approximately 1 mL of the perfusion medium running through the catheter collected</td>
</tr>
<tr>
<td>3</td>
<td>The end of the catheter and outlet tubing connector were swabbed with a steret before rejoining and the system monitored to ensure no leaks occurred</td>
</tr>
<tr>
<td>4</td>
<td>The collected effluent was vortexed and 200 µL plated onto SBA or CLED. If the sample showed turbidity, 1:10 dilutions were made as appropriate</td>
</tr>
<tr>
<td>5</td>
<td>Plates were incubated at 37°C for up to 72 hours</td>
</tr>
<tr>
<td>6</td>
<td>If growth was detected, bacterial counts were recorded as cfu/mL</td>
</tr>
<tr>
<td>7</td>
<td>If there was no growth, plates were re-incubated for a further 24 - 48 hours</td>
</tr>
<tr>
<td>8</td>
<td>Samples were collected on or as near to a daily basis as possible</td>
</tr>
<tr>
<td>9</td>
<td>If no bacterial growth was shown following day 6 of sampling, the inoculation procedure was repeated each week (every 7 days) until bacterial colonisation persisted or until the 12 week point was reached</td>
</tr>
<tr>
<td>10</td>
<td>Following successful colonisation of control catheters, new ones were set up each week</td>
</tr>
<tr>
<td>11</td>
<td>If bacterial growth persisted in the antimicrobial catheter, the test was stopped and the catheter taken down</td>
</tr>
<tr>
<td>12</td>
<td>The identity of the bacteria isolated from the colonised antimicrobial catheters was checked and MICs of rifampicin, triclosan and sparfloxacin determined</td>
</tr>
<tr>
<td>13</td>
<td>At the end of the test, approximately 5 x 1 cm segments were cut from all catheters and stored in cold acetone for Scanning Electron Microscopy (SEM) analysis</td>
</tr>
</tbody>
</table>
3.2.3. Scanning Electron Microscopy

1 cm segments were taken from the top section of the antimicrobial and control catheters tested by the in - vitro challenge and cut in half. They were stored in cold acetone to fix the bacteria to the catheter lumens. Catheter segments were removed from the acetone and dried using tetramethylsaline (Sigma-Aldrich). Samples were mounted onto 12.5 mm diameter pin - type aluminium stubs using carbon double sided adhesive mounts. They were coated with a thin layer (20 nm) of conductive platinum in an Emitech 1050 magnetron sputter coater and examined in a Philips (FEI) XL30 Scanning Electron Microscope using an accelerating beam voltage of 10 kV utilizing the secondary electron imaging mode. Images were captured at low (X500), medium (X5000) and high (X10000 - 20000) magnifications.
3.3. RESULTS

3.3.1. Growth Curves

Figure 3.4 shows the growth curve for *E.coli* in neat TSB and in artificial urine. Growth curves for MRSA, *E.faecalis*, *K.pneumoniae* and *P.mirabilis* are given in Appendix 2. Results show that the bacteria grow at similar rates and equally well in TSB and artificial urine. TSB was therefore selected as the perfusion medium to be used in the *in-vitro* model.

![E.coli growth curve in TSB and artificial urine](image-url)

*Figure 3.4: E.coli* growth curve in TSB and artificial urine.*
3.3.2. \textit{In - Vitro} Model

3.3.2.1. Meticillin Resistant \textit{Staphylococcus aureus}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3_5.png}
\caption{\textit{In - vitro} model results showing antimicrobial (test) and plain (control) catheters challenged with MRSA. $10^5$ cfu/mL bacteria were inoculated down the lumen of the catheters and allowed 1 hour to adhere. TSB was perfused through the catheter lumens at a flow rate of 0.5 mL/hour and samples of perfusion fluid collected periodically for culture. If no surviving bacteria was detected, repeat bacterial doses were given on a weekly basis with a new control catheter being set up. The killing effect was studied over a period of 12 weeks or until bacterial colonisation occurred. All test catheters were carried out in triplicate. The graph shows control catheters became colonised with MRSA upon each challenge. MRSA attached to the antimicrobial catheter by day 22 (the 4\textsuperscript{th} challenge) but colonisation did not ensue and a 12 week duration of protective activity was achieved.}
\end{figure}

MRSA readily attached to the control catheters from day 1 of each of the weekly challenges and subsequently by sample day 6 numbers of bacteria had increased to an extent that caused the catheter to become occluded. By the 4\textsuperscript{th} challenge (day 22), MRSA became attached to the antimicrobial catheter the day following inoculation but were eradicated before the next challenge commenced. At the end of the 12
week challenge, culture results from all three test catheters were negative, making the antimicrobial catheter effective at preventing bacterial colonisation by MRSA over the 12 week course.

3.3.2.2. *Escherichia coli*

![Graph showing antimicrobial (test) and plain (control) catheters challenged with *E. coli*.](image)

**Figure 3.6**: *In vitro* model results showing antimicrobial (test) and plain (control) catheters challenged with *E. coli*. $10^5$ cfu/mL bacteria were inoculated down the lumen of the catheters and allowed 1 hour to adhere. TSB was perfused through the catheter lumens at a flow rate of 0.5 mL/hour and samples of perfusion fluid collected periodically for culture. If no surviving bacteria was detected, repeat bacterial doses were given on a weekly basis with a new control catheter being set up. The killing effect was studied over a period of 12 weeks or until bacterial colonisation occurred. All test catheters were carried out in triplicate. The graph shows control catheters became colonised with *E. coli* upon each challenge. The antimicrobial catheter prevented colonisation by *E. coli* for 12 weeks.
Culture results obtained from the antimicrobial catheters challenged with *E. coli* showed no evidence of bacterial colonisation throughout the 12 week course. The antimicrobial agents were therefore effective at preventing *E. coli* colonisation over the test period. Control catheters became readily colonised with *E. coli* upon each challenge.

### 3.3.2.3. *Klebsiella pneumoniae*

![Figure 3.7: In - vitro model results showing antimicrobial (test) and plain (control) catheters challenged with *K. pneumoniae*.](image)

$10^5$ cfu/mL bacteria was inoculated down the lumen of the catheters and allowed 1 hour to adhere. TSB was perfused through the catheter lumens at a flow rate of 0.5 mL/hour and samples of perfusion fluid collected periodically for culture. If no surviving bacteria was detected, repeat bacterial doses were given on a weekly basis with a new control catheter being set up. The killing effect was studied over a period of 12 weeks or until bacterial colonisation occurred. All test catheters were carried out in triplicate. The graph shows control catheters became colonised with *K. pneumoniae* upon each challenge. *K. pneumoniae* colonised the antimicrobial catheters following the 7th (day 43), 8th (day 50) and 9th (day 57) challenge respectively.
Test catheter 1 became colonised with *K. pneumoniae* upon the 8\(^{th}\) challenge (day 50), catheter 2 upon the 7\(^{th}\) challenge (day 43) and catheter 3 on the 9\(^{th}\) challenge (day 57). All control catheters became colonised with *K. pneumoniae* with each bacterial challenge. Samples collected from the colonised antimicrobial catheters were plated onto SBA and bacterial colonies that showed differing morphologies were subcultured, identified and MICs to rifampicin, triclosan and sparfloxacin determined as shown in Table 3.2.

Table 3.2: Sparfloxacin, triclosan and rifampicin MICs and the susceptibility of the *K. pneumoniae* isolates retrieved from the colonised *in - vitro* model antimicrobial catheters.

<table>
<thead>
<tr>
<th>Test Catheter</th>
<th>Isolate No:</th>
<th>Organism</th>
<th>Sparfloxacin</th>
<th>Triclosan</th>
<th>Rifampicin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td><em>K. pneumoniae</em></td>
<td>1</td>
<td>2</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td><em>K. pneumoniae</em></td>
<td>0.38</td>
<td>1</td>
<td>R</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>Contaminant</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td><em>K. pneumoniae</em></td>
<td>2</td>
<td>2</td>
<td>R</td>
</tr>
<tr>
<td>Original Organism</td>
<td>F2630</td>
<td><em>K. pneumoniae</em></td>
<td>0.032</td>
<td>0.19</td>
<td>R</td>
</tr>
</tbody>
</table>

Catheter 2 became contaminated possibly due to failure in aseptic technique and was therefore discounted. The MIC of sparfloxacin and triclosan for the organisms isolated from test catheters 1 and 3 were found to have increased. An MIC of 2 \(\mu g/mL\) shows signs of low level resistance. The subsequent testing of these strains in a 7 day SPTT (data not shown) with silicone discs impregnated with sparfloxacin and triclosan and the three agents combined showed a decrease in the ZI diameter, indicating the strains showed reduced susceptibility to the antimicrobial agents compared to the original strain.
3.3.2.4. *Proteus mirabilis*

Figure 3.8: *In-vitro* model results showing antimicrobial (test) and plain (control) catheters challenged with *P. mirabilis*. $10^5$ cfu/mL bacteria was inoculated down the lumen of the catheters and allowed 1 hour to adhere. TSB was perfused through the catheter lumens at a flow rate of 0.5 mL/hour and samples of perfusion fluid collected periodically for culture. If no surviving bacteria was detected, repeat bacterial doses were given on a weekly basis with a new control catheter being set up. The killing effect was studied over a period of 12 weeks or until bacterial colonisation occurred. All test catheters were carried out in triplicate. The graph shows control catheters became colonised with *P. mirabilis* upon each challenge. *P. mirabilis* colonised the antimicrobial catheters following the 8th (day 50), 11th (day 71) and 12th (day 83) challenge respectively.

Test catheter 1 became colonised with *P. mirabilis* on day 50 (8th challenge), catheter 2 became colonised on day 71 (11th challenge) and catheter 3 following the 12th challenge on day 83. Control catheters became colonised with each challenge. Samples collected from colonised test catheters were plated onto CLED agar, subcultured, identified and MIC to rifampicin, triclosan and sparfloxacin determined as shown in Table 3.3.
Table 3.3: Sparfloxacin triclosan and rifampicin MICs and the susceptibility of the *P. mirabilis* isolates retrieved from the colonised *in-vitro* model antimicrobial catheters.

<table>
<thead>
<tr>
<th>Catheter</th>
<th>Isolate</th>
<th>Organism</th>
<th>Sparfloxacin (µg/mL)</th>
<th>Triclosan (µg/mL)</th>
<th>Rifampicin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td><em>P. mirabilis</em></td>
<td>0.38</td>
<td>0.38</td>
<td>R</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td><em>P. mirabilis</em></td>
<td>0.19</td>
<td>0.19</td>
<td>R</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td><em>P. mirabilis</em></td>
<td>0.38</td>
<td>0.25</td>
<td>R</td>
</tr>
<tr>
<td>Original Organism</td>
<td>F1691</td>
<td><em>P. mirabilis</em></td>
<td>0.19</td>
<td>0.094</td>
<td>R</td>
</tr>
</tbody>
</table>

The MIC of sparfloxacin for the *P. mirabilis* isolated from catheter 1 and 3 increased by 2-fold and that of the organism isolated from catheter 2 remained the same as the original *P. mirabilis*. The MIC of triclosan was found to have increased by between 2 and 4 fold. Although the MICs increased, this was not to a resistant level. This elevation in MIC and the subsequent testing of these strains in a 7 day SPTT (data not shown) with silicone discs impregnated with the antimicrobial agents did not show any alteration in the ZI diameter relative to the parent strain.
3.3.2.5. Enterococcus faecalis

**Figure 3.9:** *In-vitro* model results showing antimicrobial (test) and plain (control) catheters challenged with *E. faecalis*. $10^5$ cfu/mL bacteria were inoculated down the lumen of the catheters and allowed 1 hour to adhere. TSB was perfused through the catheter lumens at a flow rate of 0.5 mL/hour and samples of perfusion fluid collected periodically for culture. If no surviving bacteria was detected, repeat bacterial doses were given on a weekly basis with a new control catheter being set up. The killing effect was studied over a period of 12 weeks or until bacterial colonisation occurred. All test catheters were carried out in triplicate. The graph shows control catheters became colonised with *E. faecalis* upon each challenge. All three antimicrobial catheters became colonised with *E. faecalis* by the 2nd challenge (day 8).

All three antimicrobial catheters failed to prevent colonisation of *E. faecalis* by day 8 (upon the 2nd challenge) and catheters became occluded. Control catheters became colonised with each *E. faecalis* challenge. Samples collected from colonised test catheters were plated onto SBA, subcultured, identified and MIC to rifampicin, triclosan and sparfloxacin determined as shown in Table 3.4.
Table 3.4: Sparfloxacin triclosan and rifampicin MICs and the susceptibility of the *E. faecalis* isolates retrieved from the colonised *in vitro* model antimicrobial catheters.

<table>
<thead>
<tr>
<th>Catheter</th>
<th>Isolate</th>
<th>Organism</th>
<th>Sparfloxacin</th>
<th>Triclosan</th>
<th>Rifampicin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td><em>E. faecalis</em></td>
<td>0.38</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td><em>E. faecalis</em></td>
<td>0.38</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td><em>E. faecalis</em></td>
<td>0.38</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Original</td>
<td>F2633</td>
<td><em>E. faecalis</em></td>
<td>0.38</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

No difference in the MIC of sparfloxacin, triclosan or rifampicin to the isolates obtained from the failed antimicrobial catheters was seen compared to the original *E. faecalis*.
3.3.3. Scanning Electron Microscopy

Antimicrobial catheter SEMs at failure

Control catheter SEMs at the end of the weekly challenge

(A)

(B)

(C)

(D)

(E)

(F)
Figure 3.10: SEM images of the lumens of the antimicrobial (test) and plain silicone (control) Foley catheters following repeated bacterial challenges in *in-vitro* experiments. (A) MRSA test catheter (B) MRSA control catheter (C) *E.coli* test catheter (D) *E.coli* control catheter (E) *K.pneumoniae* test catheter (F) *K.pneumoniae* control catheter (G) *P.mirabilis* test catheter (H) *P.mirabilis* control catheter (I) *E.faecalis* test catheter (J) *E.faecalis* control catheter (K) Control catheter not exposed to bacteria. Images were taken at 10000 X magnification. Image A and C show what could be remnants of dead bacteria, Image B and D to J reveal extensive 3D biofilms structures on the catheter lumens and Image K shows the surface of a control catheter in its as received state.
SEM images of the control catheters challenged with all bacteria in the in - vitro model revealed extensive colonisation and biofilm development on the catheter lumens. Antimicrobial catheters showed colonisation on the luminal surface by *K.pneumoniae, P.mirabilis* and *E.faecalis* with evidence of EPS production. At lower magnifications (data not shown) all colonised catheters revealed dense patchy networks of biofilm deposits along the catheter lumen. Image A and C of antimicrobial catheters faced with repeated challenge by MRSA and *E.coli* respectively showed no evidence of viable bacteria upon culture at the end of the 12 week challenge but SEM images revealed what is probably debris from dead biofilm and chemical residue from the perfusion of TSB over the catheter surface.
3.4. DISCUSSION

The *in vitro* model is designed to challenge the lumens of the antimicrobial catheters on a weekly basis with a high dose of bacteria and monitor its ability to prevent microbial colonisation. It is based on a variation of the two-compartmental models used in encrustation studies and has separate chambers for test and control catheters which represent the catheterised urinary tract. TSB is pumped through the catheter at a rate representative of that in the humans (0.5 mL/min) and this also allows for drug clearance. Bacteria display a characteristic four-phase pattern of growth in liquid culture. This includes the lag, log, stationary and decline phases. The lag phase is a slow period of growth in response to the bacteria adapting to their environmental conditions. This is followed by the log phase where exponential growth occurs with bacteria doubling every few minutes. As more bacteria compete, the nutrient supplies decrease and the exponential growth stops. Bacterial numbers remain stable in what is the stationary phase. The decline phase occurs when the build-up of toxic waste is such that some bacteria die whilst others adapt to a long-term survival mode. It can be seen from Figure 3.4 and Appendix 2 that bacterial growth in TSB shows a similar pattern and counts to that in artificial urine, indicating that bacteria grow as well in TSB as in the artificial urine. Because of this, the ease in preparation of the quantities required and lack of variation shown by the medium, TSB was selected for use in the *in vitro* model. It is acknowledged, however, that TSB lacks the constituents that artificial or real urine have and that antimicrobial agents could differ in activity in urine than laboratory media. It was also noted that *E. faecalis* in particular favoured growth in TSB over artificial urine. TSB was, however, deemed suitable for use in this preliminary study to give an indication to how effective the antimicrobial catheter may be in preventing bacterial colonisation.
The model is flexible and can accommodate a variety of different sized catheters and specifically looks at bacterial colonisation acquired by the intraluminal route. The route of intraluminal infection is through contaminated drainage bags and connectors therefore colonisation occurs from the drainage end of the catheter and travels towards the tip. For speed of bacterial colonisation, the *in-vitro* model challenges the catheters from the opposite direction, but it is not thought that the direction of inoculation would impact on the ability of the antimicrobial catheter to resist colonisation. *In-vivo*, CAUTIs can consist of more than one bacterial species, however, this study examined the colonisation of individual species but mixed cultures could also be used and is a point for future research. The whole system is held in a water jacket and maintained at 37°C, representative of body temperature. The catheters were challenged with bacteria weekly and surviving bacteria monitored before re-challenge up to the point of 12 weeks or until the antimicrobial catheters failed to prevent bacterial colonisation. This testing period correlates with the duration catheters remain in place for asymptomatic patients before requiring replacement.

Antimicrobial catheters challenged with *E.coli* and MRSA passed the 12 week challenge and colonisation was successfully avoided. No bacterial counts were seen upon challenging catheters with *E.coli*. Upon challenge with MRSA following the 4th (day 22) challenge bacteria became attached to the material but were eradicated by day 2 or 3 prior to the next challenge. For *K.pneumoniae* following the 8th challenge (day 50) a permanent re-growth of bacteria exceeding the initial bacterial load occurred and all 3 catheters failed to prevent colonisation by the 9th challenge (day 57). Sparfloxacin and triclosan MICs for the bacteria isolated at the end of the
experiment were higher than for the original *K. pneumoniae*. Likewise, catheters challenged with *P. mirabilis* became colonised following the 8th challenge (day 50) and all 3 catheters became colonised by the final challenge. MICs of sparfloxacin and triclosan also increased but only marginally. All 3 catheters challenged with *E. faecalis* failed to prevent colonisation on the 2nd challenge (day 8) but MICs remained the same as for the original organism.

SEM images of the control and colonised antimicrobial catheters revealed extensive 3D biofilm structures on the luminal surface. SEM images of antimicrobial catheters challenged with MRSA and *E. coli* revealed what is probably a combination of debris from aggregates of dead bacteria and chemical residue from the perfusion of TSB. This is despite the catheter according to culture techniques resisting colonisation by MRSA and *E. coli* over the 12 week duration. A possible explanation for this could be that with the catheter being subject to repeated high inoculums of bacteria, aggregates of bacteria from the culture may have attached to the surface before being killed by the antimicrobial agents. This could have left behind remnants of the dead cells which were seen as distorted cells on the SEM images but which were not detectable by culture as they were non-viable by this stage. This was confirmed by all culture plates being incubated for up to 72 hours to ensure that the antimicrobial agents had killed the bacteria. The discovery of possible remnants of dead cells could have acted as anchor points which may have encouraged bacteria to colonise the catheter surface as was seen by *K. pneumoniae, P. mirabilis* and *E. faecalis*. 

According to the resistance theory described in Section 2.1.4 by incorporating two or more drugs into a polymer may act to close the MSW as for resistance to occur two mutations are required for growth [122]. This is the case only if the drugs are released at a similar rate and ratio above the MIC required for the test organisms. The colonisation of the antimicrobial catheters by *K. pneumoniae* lead to an increase in the MIC of sparfloxacin by a factor of between 11 to 63-fold up to 2 µg/mL and that of triclosan by 5 to 10-fold, up to 2 µg/mL. Concentrations of drugs at 2 µg/mL required to be active on the organism can be considered as showing signs of low level resistance. This could suggest that either both drugs (sparfloxacin and triclosan) were being released at levels below the MIC that is active on the organism, in which case this would allow for bacterial populations to multiply, colonise the catheter and bring about the generation of new mutants that will become enriched by subsequent antimicrobial exposure. Or, if sparfloxacin and triclosan were released at different ratios then one drug may have fallen below its MIC whilst the other remained above the MIC and in the MSW upon where resistance to that drug could be enriched. If *K. pneumoniae* became resistant to one of the drugs, the catheter would be acting as a monoloaded catheter and resistance to the other drug would be likely to follow upon prolonged exposure. Even though the SPTT infers that sparfloxacin and triclosan display activity towards *K. pneumoniae* for over 100 days with no emergence of resistance, the *in-vitro* model is a dynamic system through which drug release and drug clearance is more likely to simulate that in the human catheterised urinary tract. It is therefore a more clinically predictive approach to assessing the potential for bacterial colonisation and resistance development.
The colonisation of the antimicrobial catheters by *P. mirabilis* led to a slight increase in the MIC of triclosan of between 2 to 4-fold, up to 0.38 µg/mL. Triclosan is the main agent effective against *P. mirabilis* but the increase in MIC was lower than the increase in MIC towards *K. pneumoniae* colonised catheters and would not be considered to be to resistant concentrations. The MIC of sparflloxacin to *P. mirabilis* which colonised the test catheters remained similar to the original *P. mirabilis* strain, probably because released concentrations were only active on *P. mirabilis* only over a short period according to SPTT results. If the *in-vitro* experiment was not stopped as soon as the antimicrobial catheters became colonised with bacteria, it is likely that more bacteria would mutate upon the prolonged exposure to triclosan resulting in higher MICs. It is possible that triclosan concentrations were too low to prevent the progression of *P. mirabilis* colonisation, however an 8 week duration of protective activity without signs of resistance was achieved and drug release studies are required to assess concentrations of drugs being released.

Antimicrobial catheters became colonised with *E. faecalis* upon the 2nd challenge (day 8). This corresponds well with the short duration of activity shown by the SPTT and indicates that the drugs do not possess sufficient activity to prevent colonisation by *E. faecalis* over prolonged periods. Appendix 2 also shows that *E. faecalis* grew better in TSB than artificial urine which could act in favour of the growth of *E. faecalis* making the *in-vitro* model challenge more difficult. The antimicrobial catheters lack of ability to prevent *E. faecalis* colonisation over a prolonged duration could be of concern but the clinical implication of it in terms of symptomatic CAUTI requires further investigation.
The *in-vitro* model has shown that the antimicrobial catheter is most effective in preventing colonisation caused by the MRSA and *E.coli* strains studied. It also showed protective activity against colonisation by *K.pneumoniae* and *P.mirabilis* for a minimum of 50 days but was not active against *E.faecalis* over the long-term. The antimicrobial catheter has shown it is able to prevent bacterial colonisation caused by certain organisms associated with CAUTI over a prolonged course, however, drug release information is required to ascertain whether concentrations are sufficient to affect those bacteria which colonised the catheter.
CHAPTER 4

DRUG CONTENT AND RELEASE
4.0. DRUG CONTENT AND RELEASE

4.1. INTRODUCTION

Administration of systemic antibiotics can result in concentrations at the site of infection that are below desired therapeutic levels and many therapies tested in animal models which have been effective, are at doses greater than could safely be applied in humans [175]. The concept of local drug controlled - delivery devices is one of great interest. There are a variety of definitions used to describe controlled delivery systems, but all refer to systems that consist of a drug and a drug carrier. The purpose of drug delivery systems is to deliver drugs locally to the site of infection/injury within an effective therapeutic range which avoids systemic toxicity [176]. A variety of biomaterials, for example, polymeric materials have been used as drug carriers. As for urinary catheters, silicone is an example of a material in which liquids, gases and drug particles can easily penetrate. Work has led to the conclusions that low molecular weight and lipophilic drugs easily diffuse into silicone [176]. The ease with which substances can penetrate silicone is due to its structure. The large atomic volume of the silicon atom as well as the size and position of the constituent groups leads to rotation around the Si-O-Si bonds [176]. Silicone polymers form helices and the silicon-oxygen bond angles create large amounts of free volume in silicone elastomers and this is responsible for their permeability to certain liquids, gases and drugs [176].

Drug release encompasses several processes that contribute to the transfer of drug from the carrier to the bathing solution. Drug release can occur by diffusion, degradation/erosion, dissolution or swelling [176]. Depending on the system, the
release may occur through any or all of these mechanisms. Drug release data can be analyzed to determine the drug release mechanism by fitting the data into mathematical models as shown in equations 4.1 to 4.5. The graphical presentation of those can be used to evaluate the mechanism of drug release, with the model that best fits the release data being selected on the basis of the highest correlation coefficient (r value).

**Equation 4.1:** Zero Order \[ Qt = Q_0 + K_0t \]

**Equation 4.2:** First Order \[ \log Qt = \log Q_0 + Kt/2.303 \]

**Equation 4.3:** Higuchi \[ Qt = K_{Ht}^{1/2} \]

**Equation 4.4:** Hixson-Crowell \[ \text{cube root } Q_0 – \text{cube root } Qt = K_{HC}t \]

**Equation 4.5:** Korsmeyer-Peppas \[ F = (Mt/M) = K_mt^n \]

Where: \( Qt \) = cumulative amount of drug released at time \( t \), \( Q_0 \) = initial amount of drug, \( t = \) time, \( K_0, K, K_{H}, K_{HC}, K_m = \) kinetic release constants, \( F = \) fraction of drug released at time \( t \), \( Mt = \) amount of drug released at time \( t \), \( M = \) total amount of drug in dosage form, \( n = \) diffusion or release exponent

To determine the highest correlation coefficient, the following plots can be made: Cumulative % drug release vs time (zero order), log cumulative of % drug remaining vs time (first order), cumulative % drug release vs square root time (Higuchi), cube root initial drug concentration minus cube root cumulative % remaining in matrix vs time (Hixson-Crowell) and log cumulative % drug release vs log time (Korsmeyer-Peppas) [177].
A brief description of each drug release mechanism is given below:

- **Zero order**: describes systems where the drug release rate is independent of its concentration of the dissolved substances. There is no time lag or burst effect over prolonged periods of time.

- **First order**: the drug release rate depends on its concentration.

- **Higuchi**: this model suggests that the drugs are released by diffusion. It describes the release of drug/s from an insoluble matrix as a square root of time - dependent process based on the Fickian diffusion equation.

- **Hixson-Crowell**: is a cube root law - based model which describes drug release by dissolution and from systems where there is a change in surface area and diameter of particles.

- **Korsmeyer-Peppas**: describes drug release from a polymeric system. To establish the mechanism of drug release, the first 60% of release data is fitted into the Korsmeyer-Peppas equation. The n value (slope of the line) is used to characterise the release mechanism for cylindrical shaped matrices.

\[
\begin{align*}
n &= 0.45 \text{ Fickian diffusion} \\
n &= 0.45 < n < 0.89 \text{ Anomalous (non-Fickian) diffusion} \\
n &= 0.89 \text{ Case-II transport} \\
n &= > 0.89 \text{ Super case-II transport}
\end{align*}
\]

Anomalous diffusion or non Fickian diffusion refers to a combination of both diffusion - and erosion - controlled rate release. Case-2 relaxation or super case transport-II refers to the erosion of the polymeric chain.
Usually if the carrier system is silicone rubber, the release of a drug occurs by the diffusion mechanism [176]. Drug release through a matrix type controlled release device can be described by the classical Fickian diffusion theory. According to Fick’s law, the release of drug from a system is non-linear due to the increase in diffusional length resistance and/or the decrease in the inwardly releasing surface area with time [178]. There are a number of ways that drug release can be measured, for example, by ultra violet spectroscopy (UV-Spec) or high performance liquid chromatography (HPLC).

4.1.1. High Performance Liquid Chromatography

High Performance Liquid Chromatography (HPLC) is a chromatographic technique used to separate, identify and quantify compounds in a mixture. It is an analytical technique widely used on samples with components ranging from small organic and inorganic molecules and ions to polymers and proteins with high molecular weights. A schematic diagram of a basic HPLC system is shown in Figure 4.1.
The solvent delivery system (pump) delivers the mobile phase which typically consists of a water, methanol or an acetonitrile mix through the system at a specified flow rate. The injector places the sample into the flowing mobile phase for introduction onto the column. Most analytical columns are 10, 15 or 25 cm in length with internal diameters of 4.6 or 5 mm [180]. They are generally filled with 3, 5 or 10 μm packing material such as silica. Many HPLC separations are performed in the reverse-phase mode (RP-HPLC). RP-HPLC uses a non polar stationary phase and a polar mobile phase. Analytes are separated based on their relative solubility between two liquid phases and are eluted from the column in order of increasing hydrophobicity (decreasing polarity) [180]. The more polar or hydrophilic a compound is the faster it will elute from the column as it will travel with the mobile phase and the more nonpolar or hydrophobic an analyte is the longer it will be retained on the column through interaction with the non polar stationary phase. To achieve separation of sample components isocratic or gradient elution can be used.
In isocratic elution, solvent composition and flow rate are held constant. By varying the mobile phase composition or flow rate, this gradient type elution serves to elute components in a mixture that have varying polarities quicker than in isocratic elution. A detector translates concentration changes in the column effluent into electrical signals. The most widely used detectors are based on ultraviolet-visible (UV-Vis) and fluorescence spectrophotometry, refractive index determination and electrochemical analysis. A commonly used type of UV-Vis absorption detector is the diode array spectrophotometric detector of variable wavelength. The detector response, in the form of an electrical signal can be recorded (the chromatogram) and used for qualitative and quantitative analysis via analyte retention times and peak areas [180].

4.1.2. Objectives

The aim of this chapter is to use RP-HPLC to (A) determine the total content of each antimicrobial agent within the antimicrobial urinary catheter and (B) to determine the concentrations of antimicrobial agents released over a 28 day period. By investigating this, the concentration and ratios of drugs being release can be used to establish if sufficient concentrations are present to deliver a long lasting effect and if the concentrations are sufficient to reduce the opportunity for bacterial colonisation.
4.2. METHODS

4.2.1. High Performance Liquid Chromatography Protocol

1 cm segments were cut from silicone Foley catheters and impregnated with rifampicin, sparfloxacin and triclosan as described in section 2.2.4. RP-HPLC analysis was performed on an Agilent 1090 HPLC machine with a diode-array variable wavelength UV detector (HPLC - UV) (Agilent Technologies, Berkshire, UK) connected to a Chemstation operating software system. Chromatographic separations were performed on an Eclipse XDB-C8 (5 µm, i.d. 4.6 mm x 150 mm) column (Agilent Technologies). Mobile phase was a mixture of aqueous sodium dihydrogen phosphate (15 mM, pH 2.5) (Sigma-Aldrich) with 10% acetonitrile (ACN) (Fisher Scientific, Loughborough, UK): methanol (MeOH) (Fisher Scientific) used after filtration through a 0.45 µm nylon membrane filter (Whatman, Dassel, Germany) and degassed. Drugs were eluted using a gradient elution profile of methanol ranging from 58% to 90%. A flow rate of 1 mL/min and temperature of 40°C was employed and UV detection at 254 nm for rifampicin, 290 nm for sparfloxacin and 279 nm for triclosan. Retention times were approximately 1.5, 4.0 and 8.7 minutes for sparfloxacin, rifampicin and triclosan respectively.
4.2.2. Total Antimicrobial Content

4.2.2.1. Calibration Curve for the Determination of the Total Drug Content in Catheter Segments

To cover the expected drug range a series of at least five different concentrations of each antimicrobial agent was made. 0.01 g of rifampicin, 0.04 g of sparflloxacin and 0.035 g of triclosan were accurately weighed and dissolved in ACN: HPLC grade water (Fisher Scientific): MeOH in a 1:3:6 ratio to a total of 100 mL. Serial dilutions were made in 1:3:6 ratio of ACN: water: MeOH having a concentration of 5, 10, 15, 20 and 25 µg/mL for rifampicin, 10, 50, 100, 200, 300 and 400 µg/mL for sparflloxacin and 10, 50 100, 200, 300 and 350 µg/mL for triclosan. Concentrations were filtered through a 25 mm x 0.2 µm syringe filter (Sartorius Stedim Biotech, Goettingen, Germany) into 2 mL amber HPLC vials (Kinesis, Cambridgeshire, UK) and 5 µL injected into the column. All injections were carried out in triplicate.

4.2.2.2. Total Drug Content in Catheter Segments

1 cm catheter segments containing rifampicin, sparflloxacin and triclosan were placed into glass vials containing 2 mL chloroform. The chloroform acts to expand the silicone matrix allowing the antimicrobial agents to be extracted. Segments were left in chloroform at room temperature for 24 hours. Catheter segments were then placed into new glass vials containing a further 2 mL of chloroform whilst the chloroform from the previous vial was left to evaporate at room temperature. The process was repeated a total of three times to ensure that as much of the antimicrobial agents were extracted as possible. All chloroform was pooled and evaporated to leave the drug
residue. The drug residue was dissolved in ACN: HPLC grade water: MeOH in a 1:3:6 ratio to a total of 1 mL, filtered through a 4.0 mm x 0.2 μm syringe filter (Sartorius Stedim Biotech) into amber HPLC vials and 5 μL injected into the column. All experiments were carried out in triplicate.

4.2.3. Drug Release Studies

4.2.3.1. Calibration Curve for the Determination of Drugs Released from Catheter Segments

0.01 g of sparfloxacin and triclosan were accurately weighed and dissolved in ACN: HPLC grade water: MeOH in a 1:3:6 ratio to a total of 100 mL. Serial dilutions were made in 1:3:6 ratio of ACN: water: MeOH having a concentration ranging from 5, 10, 20, 40 and 60 μg/mL for sparfloxacin and 5, 10, 20, 30 and 40 μg/mL for triclosan. Concentrations were filtered through a 25 mm x 0.2 μm syringe filter into amber HPLC vials and 100 μL injected into the column. All injections were carried out in triplicate.

4.2.3.2. Drug Release from Catheter Segments

1cm catheter segments containing all three antimicrobial agents and catheter segments without antimicrobial agents as controls were placed into glass vials containing 2 mL HPLC grade water (pH7) in a 37°C incubator with constant agitation. Segments were transferred daily or every 2 - 4 days into fresh 2 mL water to create nearly perfect sink conditions. Elutions were collected and stored in a
-80°C freezer until required for HPLC analysis. Drug release studies were carried out over 28 days and all tests were performed in triplicate.

Elutions were concentrated by liquid - liquid extraction using chloroform. 2 mL of chloroform was added to the water in which the drugs had been released and shaken for 20 - 30 seconds. Both phases were allowed to separate. The organic phase was collected and further chloroform added to extract any further drug from the water. The two volumes of chloroform were pooled and evaporated at room temperature to leave the drug residue. Drug residues were dissolved in ACN: HPLC grade water: MeOH in a 1:3:6 ratio to a total of 200 µL and filtered through a 4 mm x 0.2 µm syringe filter into amber HPLC vials. 100 µL was injected into the column.

4.2.3.3. Percentage Recovery Experiment

10 and 20 µg/mL of sparfloxacin and triclosan were made up in chloroform and concentrated by liquid - liquid extraction. Drug residues were dissolved in ACN: HPLC grade water: MeOH in a 1:3:6 ratio to a total of 200 µL and filtered through a 4 mm x 0.2 µm syringe filter into amber HPLC vials. 100 µL was injected into the column and all experiments were performed in triplicate. Peak areas were compared to a series of known sparfloxacin and triclosan concentrations and the % of drug recovered following liquid - liquid extraction determined.
4.3. RESULTS

4.3.1. Total Antimicrobial Content

Figure 4.2 shows a typical chromatogram of rifampicin, triclosan and sparfloxacin and Figure 4.3 shows the calibration curves used to determine the total antimicrobial content extracted from the silicone segments.

Figure 4.2: HPLC chromatograms of (A) rifampicin at 254 nm, (B) sparfloxacin at 290 nm and (C) triclosan at 279 nm. Peaks with a * represent the antimicrobial agents.
Figure 4.3: Graph depicting the standard calibration curve of various concentrations of (A) rifampicin (B) sparfloxacin (C) triclosan plotted against the peak area as determined by HPLC - UV. Each value represents the average of three tests with standard deviations. The standard curves were used to determine the total antimicrobial content in catheter segments.
Table 4.1: Peak area of the total amount of rifampicin, triclosan and sparfloxacin extracted from catheter segments as determined by HPLC - UV. Average of three segments with standard deviation and coefficient of variation are reported.

<table>
<thead>
<tr>
<th>Segment</th>
<th>Rifampicin</th>
<th>Triclosan</th>
<th>Sparfloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>89.76</td>
<td>903.35</td>
<td>4259.31</td>
</tr>
<tr>
<td>2</td>
<td>72.55</td>
<td>844.99</td>
<td>3373.52</td>
</tr>
<tr>
<td>3</td>
<td>73.35</td>
<td>934.87</td>
<td>3704.34</td>
</tr>
<tr>
<td>Average</td>
<td>78.55</td>
<td>894.40</td>
<td>3779.06</td>
</tr>
<tr>
<td>StDev</td>
<td>9.71</td>
<td>45.6</td>
<td>447.6</td>
</tr>
<tr>
<td>CV</td>
<td>0.12</td>
<td>0.05</td>
<td>0.12</td>
</tr>
</tbody>
</table>

To establish the total concentration of each drug extracted from the catheter segments the equation from the calibration curves shown in Figure 4.3 were implemented. Where:

**Equation 4.6:** \( y = mx + c \) therefore \( x = y - c/m \)

\( y = \text{peak area (from Table 4.1)}, \ m = \text{gradient of the straight line}, \ x = \text{concentration of drug (µg/mL)}, \ c = \text{y intercept} \)

To establish the total drug content per catheter as shown in Table 4.2, the total weight of a catheter (8.47 g) was divided by the average weight of 1 cm catheter segments (0.1346 g) and the sum (62.93) multiplied by the drug content per catheter segment.
### Table 4.2: Total drug content per catheter and % w/w per catheter.

<table>
<thead>
<tr>
<th>Drug content in catheter segment (µg/cm) or (µg/mL)</th>
<th>Rifampicin</th>
<th>Triclosan</th>
<th>Sparfloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug content per catheter (mg)</td>
<td>0.49</td>
<td>14.00</td>
<td>13.25</td>
</tr>
<tr>
<td>% drug per catheter (w/w)</td>
<td>0.006</td>
<td>0.17</td>
<td>0.16</td>
</tr>
</tbody>
</table>

By exposing silicone to chloroform containing 1% triclosan and 1% sparfloxacin, 14 mg of triclosan and 13.25 mg of sparfloxacin were incorporated into the catheter, assuming all was extracted. Of the 0.2% rifampicin, 0.49 mg representing 0.006% of the total weight of a catheter was incorporated.

### 4.3.2. Drug Release

The quantity of rifampicin, sparfloxacin and triclosan released from the catheter segments was studied over 28 days and is shown in Table 4.3. Drug extracts required concentration for detection and the percentage of each drug recovered from the liquid - liquid extraction process determined. 98.1% of sparfloxacin and 98.8% triclosan were recovered. Concentrations of rifampicin being released were too low to detect using the HPLC protocol outlined.
Table 4.3: Concentrations of sparfloxacin and triclosan (µg/catheter) released from the antimicrobial urinary catheter over an elution period of 28 days. Each value represents the average of three replicates with standard deviations.

<table>
<thead>
<tr>
<th>Day</th>
<th>Sparfloxacin release µg/catheter</th>
<th>Triclosan release µg/catheter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>506.01 ± 80.18</td>
<td>320.42 ± 120.23</td>
</tr>
<tr>
<td>2</td>
<td>207.45 ± 127.00</td>
<td>329.60 ± 80.71</td>
</tr>
<tr>
<td>3</td>
<td>198.42 ± 50.72</td>
<td>383.05 ± 43.71</td>
</tr>
<tr>
<td>4</td>
<td>126.88 ± 55.58</td>
<td>318.72 ± 107.48</td>
</tr>
<tr>
<td>5</td>
<td>121.08 ± 22.43</td>
<td>381.55 ± 22.84</td>
</tr>
<tr>
<td>6</td>
<td>100.47 ± 6.42</td>
<td>295.06 ± 22.58</td>
</tr>
<tr>
<td>8</td>
<td>188.62 ± 28.12</td>
<td>349.31 ± 8.54</td>
</tr>
<tr>
<td>12</td>
<td>209.59 ± 75.43</td>
<td>299.06 ± 36.48</td>
</tr>
<tr>
<td>14</td>
<td>161.84 ± 36.46</td>
<td>269.60 ± 83.39</td>
</tr>
<tr>
<td>19</td>
<td>136.62 ± 15.04</td>
<td>286.07 ± 19.77</td>
</tr>
<tr>
<td>21</td>
<td>194.01 ± 84.14</td>
<td>295.49 ± 80.75</td>
</tr>
<tr>
<td>24</td>
<td>143.53 ± 15.83</td>
<td>195.01 ± 53.98</td>
</tr>
<tr>
<td>26</td>
<td>150.28 ± 33.23</td>
<td>250.06 ± 46.17</td>
</tr>
<tr>
<td>28</td>
<td>120.47 ± 13.25</td>
<td>218.07 ± 23.04</td>
</tr>
</tbody>
</table>

A total of 2.6 mg sparfloxacin and 4.2 mg triclosan was released over 28 days representing 19.8% and 29.9% of the total content. This gives a sparfloxacin release of approximately 0.07 mg/catheter/day following the initial burst effect (or 1.25 µg/mL/day) and 0.15 mg/catheter/day (or 2.4 µg/mL/day) of triclosan over the 28 day course. (Refer to Appendix 3 for sparfloxacin and triclosan release as µg/mL).

Extractive methods can give results with more variation which may be due to a loss of pharmaceutical substance during handling [181], which may be the reason why error margins are large and variable. Figure 4.4 shows the drug release data as cumulative percentage release over the 28 days.
Drug release data were analyzed to determine the drug release mechanism by fitting the release data into the mathematical models shown in equations 4.1 to 4.5. The model with the highest correlation coefficient shown from the graphical presentation of the data was used to select the type of model that best fitted the release data. $R^2$ values are given in Table 4.4.
Table 4.4: $R^2$ linear regression values for results from drug release data fitted into mathematical models to determine the mechanism of drug release from silicone catheter segments.

<table>
<thead>
<tr>
<th>Model</th>
<th>Sparfloxacin</th>
<th>Triclosan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero Order</td>
<td>0.9568</td>
<td>0.9287</td>
</tr>
<tr>
<td>First Order</td>
<td>0.9653</td>
<td>0.9487</td>
</tr>
<tr>
<td>Higuchi</td>
<td>0.9923</td>
<td>0.9834</td>
</tr>
<tr>
<td>Hixson-Crowell</td>
<td>0.9626</td>
<td>0.9425</td>
</tr>
<tr>
<td>Korsmeyer-Peppas</td>
<td>0.9916</td>
<td>0.9523</td>
</tr>
</tbody>
</table>

The $R^2$ values show that the drug release data fit well to the Higuchi model expression, suggesting that triclosan and sparfloxacin are released from the silicone by a diffusion controlled process. A plot of the Higuchi model is given in Figure 4.5.

Figure 4.5: Drug release data fitted into the Higuchi equation of cumulative % drug release versus the square root of time.


4.4. DISCUSSION

HPLC studies revealed from the chloroform solution containing 0.2% rifampicin, 1% sparfloxacin and 1% triclosan used in the impregnation process, 0.49 mg of rifampicin, 13.25 mg of sparfloxacin and 14 mg of triclosan became incorporated into the urinary catheter. On a w/w basis a catheter contains 0.006% rifampicin, 0.17% triclosan and 0.16% sparfloxacin. The total drug content in a catheter compared to the content remaining in the immersion solution and the efficiency of drug uptake was not established as the total amount of each antimicrobial agent in the chloroform solution was not determined but is a point for future studies.

As reported in Sections 2.1.5.1 and 2.1.5.2 a typical oral dose of rifampicin consists of 150 - 300 mg, reaching peak serum concentrations ranging from 4 - 32 µg/mL (average 7 µg/mL) [182] which is well tolerated and 400 mg reducing to 200 mg per day of sparfloxacin, reaching peak serum concentrations of 1 - 1.5 µg/mL [183]. The total amount of these drugs released from the catheter over a 24 hour period is below a single therapeutic dose and due to the slow release into the localised surroundings it is envisaged that issues of drug intolerance and organ toxicity will not been seen. It is also possible that, due to the vast use of triclosan in everyday consumer products, individuals could well be exposed to higher usage than the concentrations released from the catheter. Calafat et al (2008) [184], analysed the urine of 2,517 people aged >6 years from the US general population and found it contained concentrations of triclosan ranging from 2.4 - 3790 µg/L. Consumer soaps can typically contain 0.1 - 0.25% [185] triclosan and deodorant sprays/anti-perspirants between 0.15% and 0.25% w/v [186]. The urinary catheter releases a total of <400
µg of triclosan a day (or 2.4 µg/mL/day) into the surrounding vicinity and the urine, making toxicity unlikely. Based on what is known there is not sufficient evidence to suggest that triclosan is a threat to human health, though a better understanding of potential health effects in respect to its wide use in consumer products is required. Due to the high lipid solubility of triclosan questions have been raised regarding its distribution properties and accumulation in the body [187]. There have however been reports [157] of triclosan incorporated into peritoneal dialysis catheters that were exposed to the sensitive perineum in mice and no gross lesions upon necroscopy at 7 or 30 days were seen. Only minimal inflammation which was associated with the surgical process was reported [157]. Triclosan has also been bonded to vascular prostheses and in animal studies found not to cause a more pronounced inflammatory response than commonly used untreated vascular prostheses [188], making it suitable for use in patients at risk of infection. Studies such as these have shown in animal studies that triclosan released from medical devices do not lead to local or systemic irritation and toxicity. The \textit{in vitro} model has given an indication of the likely effect against bacterial colonisation and the drug studies revealed the quantities that are being released but in addition, to satisfy concerns over the use of triclosan long-term, further long-term \textit{in vivo} studies may be required.

Efficacy of a device strongly depends on the rate and manner in which drugs are released. This is dependent on the host matrix the drug is loaded into, the type of drug and drug clearance rate. If drugs are released too quickly all could be released before infection is arrested and if release is delayed the infection may further set in. The release pattern observed by sparfloxacin (Table 4.3 and Appendix 3) was
bisphasic, characterised by an initial burst effect followed by a slower sustained release. This could be important at the high risk time during catheter insertion to prevent bacterial colonisation. Triclosan showed a more even release that gradually declined over the 28 day period (Table 4.3 and Appendix 3). This correlates well with the duration of activity trend displayed by the SPTT (Figure 2.6 and 2.7) for both sparfloxacin and triclosan. Rifampicin release was not detected as concentrations were too low but the SPTT (Figure 2.5) suggests that the pattern of release would follow a biphasic trend. The total content of rifampicin in the catheter (0.006% w/w) was substantially less than sparfloxacin and triclosan which may have been why upon the release of rifampicin from the silicone the low concentrations were not detected. To detect the presence of analytes at very low levels, more sensitive detection techniques such as fluorescence could be employed. The main attraction of fluorescence detection is that for strongly fluorescent molecules, it can offer limits of detection two or three orders of magnitude lower than UV absorbance levels [189]. A higher concentration of rifampicin in the chloroform equivalent to sparfloxacin and triclosan may have enabled the drug to be detected on its release. A greater total content and release of rifampicin may have also acted to have a more powerful killing effect on the Gram positive bacteria. Liang et al (2007) [190] reported on silicone shunts containing 8.3% w/w rifampicin that showed a high initial release rate followed by a relatively constant release rate for a further 90 days. They reported a drug release mechanism that showed linearity against $t^{0.5}$ (square root of time). Mechanical properties of the silicone shunt were however not tested and it may be that at drug levels this high mechanical performance could be adversely affected.
Drug release data were fitted into the mathematical models as shown in equations 4.1 to 4.5 to confirm the type of drug release mechanism. Based on the interpretation of the correlation coefficient, the best fit (0.9834 for triclosan and 0.9923 for sparfloxacin) was observed from the Higuchi equation, which suggests that the drugs are released by a diffusion process. This agrees with previous reports [176, 191] that agents released from matrix-loaded devices is via diffusion. The mathematical modelling of release from diffusion-controlled systems relies on the fundamental Fick’s law, which describes the transport of molecules by a concentration gradient. Fickian drug release is characterised by a linear dependence of the released drug with the square root of time that is concentration-dependent. It suggests that diffusion of molecules through a system decreases with time due to the extended distance that the drugs must diffuse through the matrix to the exterior [192]. Diffusion occurs as drug molecules inside the matrix move from one site to another as a result of random molecular motion. For this to happen a system should be sufficiently dilute so that diffusion is not impeded by approach to saturation of the compound of interest (forming ideal sink conditions). If the new site is occupied, the move is rejected, and the particle is removed from the lattice as soon as it migrates to a free site [193]. At the surface of a catheter, the drugs diffusing out become highly concentrated within the so-called stagnant diffusion layer (Nernst layer). Underlying this shows a sustained release of sparfloxacin and triclosan.

Kohnen et al (2003) [118], found that an equal release ratio was achieved from ventricular catheters impregnated with 1.4 wt% of rifampicin and 0.6 wt% of sparfloxacin. It showed a high release rate in the initial phase dropping to lower amounts with time although the ratio between the antibiotics remained constant for at
least 40 days. It is important to release the same ratio of drugs as excess elution of one drug compared to the other can lead to a catheter acting as a mono-loaded device in which bacterial resistance can emerge. The release ratio of sparfloxacin and triclosan (19.8% and 29.9% respectively) over the 28 day period was found to differ and the low concentrations of rifampicin released did not allow assessment of levels corresponding to the MIC of susceptible organisms. As the MIC of triclosan and sparfloxacin were found to increase following repeated challenges with *K.pneumoniae* and *P.mirabilis* during the *in-vitro* model challenge, it suggests that the concentrations of antimicrobial agents being released despite the MIC towards the organisms were not sufficient to prevent bacterial colonisation. A study by Schierholz et al (1997) [194], incorporated a much higher content of antimicrobial agents (5 wt% rifampicin, 1.7 wt% fusidic acid and 1.4 wt% mupirocin) into a silicone catheter using a diffusion process and they report that they could not demonstrate bacterial resistance to the combination of drugs using gradient plates or killing experiments. The antimicrobial urinary catheter may therefore have a greater effect on reducing bacterial colonisation and potential for resistance development if drug concentrations were increased and adjusted to release at equal ratios and more data could be gathered if drug release studies were taken to the end point of 12 weeks rather than 28 days. Further investigation and optimisation of drug content and release is required to enable this to happen.
CHAPTER 5
SURFACE ANALYSIS
5.0. SURFACE ANALYSIS

5.1. INTRODUCTION

Biomaterials used in the production of medical devices will at some stage interface with the environment of the body, with which they must be biocompatible. Biocompatibility has been defined as the ‘utopian state where a biomaterial presents an interface with a physiologic environment without the material adversely affecting that environment or the environment adversely affecting the material’ [195]. Understanding how a biomaterial interacts with the physical environment and how changes to materials, like the incorporation of drugs, or the development of new materials would help to determine whether new materials are more biocompatible than existing ones and better able to prevent some of the complications when implanted into the human body. There are currently no standardised methods for comprehensively evaluating new biomaterials, but there is a string of scientific evidence towards using surface analysis techniques for studying and characterising biomaterials [196]. It is believed that most complications associated with medical devices take place at the surface of the device as this is the region that interfaces between the biomaterial and the biological environment and that differences in biomaterial surface characteristics such as roughness and hydrophobicity may be possible reasons for why one biomaterial is less prone to bacterial adherence than another [77]. The surface of a biomaterial is only a few atomic layers deep and it is the surface composition and structure of a biomaterial that triggers the sequence of biological events that occurs when a biomaterial is placed in the body [197]. As the surface composition and structure of a biomaterial can be different from its bulk.
composition and structure, surface - sensitive techniques are required [197]. Over the past 20 years there has been a rise in the number of techniques capable of probing biointerfaces [198]. There is a large array of surface analytical techniques each designed with unique applications in mind. These may entail determining the composition, structure and distribution of chemical species present on a biomaterials surface as well as topographical features of a surface and the likely interaction of solid surfaces with external environmental fluids (hydrophobic and hydrophilic effects).

As there are currently no biomaterials used for urinary catheters that are completely biocompatible with the environment they are placed, it is important to gather an understanding of the relationship between surface properties and biological performance and to characterise the biomaterial surface in detail. Section 5.1.1 to 5.1.4 details the surface analytical techniques used in this study and the purpose of their use.

5.1.1. X-Ray Photoelectron Spectroscopy

X-ray Photoelectron Spectroscopy (XPS) is a technique commonly used by surface analysts to identify and characterise the chemistry of surfaces, to characterise and optimise surface modifications and to characterise biological interactions especially the adsorption of proteins and other biomolecules at interfaces [198]. The components necessary to perform XPS are, an X-ray source, an electron energy analyser, a detection system and a sample stage, all maintained under ultra - high vacuum. XPS involves the irradiation of a sample’s surface by an X-ray beam. This leads to the adsorption of X-rays by the atoms on the surface and the ejection of core
and valence electrons (photoelectrons). Photoelectron emission occurs in all atoms in which core shell electrons have a binding energy (energy difference between the ground state and Fermi state of the electron) that is lower than the X-ray energy [199]. The kinetic energy of the emitted electrons is analyzed and the binding energy determined as described in the relationship shown by Equation 5.1.

**Equation 5.1:** \( E_B = h\nu - E_K - \phi \)

Where: \( E_B \) = the energy binding the electron to the atom, \( h\nu \) = energy of the x-ray, \( E_K \) = kinetic energy of the emitted electrons, \( \phi \) = work function.

A spectrum of binding energy peaks are produced each having energies that are unique to a specific atomic element and sensitive to the chemical state of the element. The intensities of the photoelectrons are proportional to the concentration of the element they are ejected from. As the free path of photoelectrons is relatively short, only electrons emitted from the first 10 nm of a material reach the detector, making it a highly specific surface analysis technique [199].

### 5.1.1.1. X-Ray Photoelectron Spectroscopy Objective

The impact that conditioning films on medical device surfaces have upon bacterial colonisation is important and was taken into account during the adherence studies (tk100) in Chapter 2. To determine whether an hour exposure to urine was sufficient time for catheter surfaces to become coated with a urinary conditioning film was assessed using XPS. The adsorption of protein elements from the urine and other urinary components to the surface was used to determine this.
5.1.2. Atomic Force Microscopy

Atomic Force Microscopy (AFM) was invented in 1986 and is a technique that produces high resolution images of the surface topography of a sample [200]. It operates by scanning the surface of a material using a very sharp tip (probe) which is commonly made from very fine silicon or silicon nitride in the shape of a pyramid and is placed on the end of a silicon or silicon nitride cantilever [201]. The cantilever is mounted on a piezoelectric ceramic scanner, usually in the form of a hollow cylinder which scans over the surface of a sample [202]. As the tip is brought close to a surface or is retracted from it, different forces e.g. van der Waals forces either attract or repel the tip and vertical displacement of the cantilever occurs. A laser spot reflected from the top surface of the cantilever into an array of photodiodes is typically used to measure the deflection. The forces depend on the nature of the sample, the distance between the probe and sample, the probe geometry and contamination of the sample surface. These deflections are recorded and processed using imaging software to create a three dimensional topographical image of the surface. Figure 5.1 illustrates the main components of an AFM.
Atomic force microscopy can operate under two principal modes namely contact and tapping modes. In contact mode AFM the cantilever is in constant contact with the surface and the tip is dragged over the surface and contours measured. However, samples and the tip can be easily damaged using this mode and images can be distorted. In tapping mode AFM, cantilevers are oscillated with probes tapping the surface at speeds of 50,000 to 500,000 cycles/sec [203]. As the tip passes over a bump in the surface the cantilever has less room to oscillate and the amplitude is decreased. As it passes over a depression the cantilever has more room to oscillate and the amplitude increases. More recently, a new mode of AFM known as PeakForce Quantitative NanoMechanical Mapping (QNM) was introduced. This is also based on tapping technology but keeps indentations small to deliver non-destructive, high resolution images which has allowed for samples from soft gels to rigid polymers to be analysed. PeakForce AFM can map nanomechanical properties,
including modulus and adhesion whilst imaging sample topography i.e. surface roughness (commonly described as the root mean square).

5.1.2.1. Atomic Force Microscopy Objective

Several studies have reported that an increase in surface roughness can promote bacterial adherence and subsequent colonisation [204, 205]. In this study, AFM was used to compare the surface topography (roughness) of the antimicrobial urinary catheters with the conventional (control) urinary catheter. The effect on surface roughness as the antimicrobial catheters were immersed in an aqueous environment for 12 weeks to simulate drug release was also investigated to establish if there was any increase or decrease in the surface roughness due to elution of the drugs and if this may have any impact on bacterial colonisation.

5.1.3. Time of Flight - Secondary Ion Mass Spectroscopy

Time of Flight - Secondary Ion Mass Spectroscopy (ToF-SIMS) is widely used by surface analysts in the characterisation of biomaterial surfaces. A schematic diagram illustrating components of a ToF-SIMS analyser is depicted in Figure 5.2.
Primary ion sources commonly used in ToF-SIMS include caesium, gallium and bismuth [206]. The surface of a sample is bombarded with primary ions as the ion beam is pulsed over it for short periods. Collisions between primary ions and atoms of a surface are highly energetic resulting in extensive fragmentation and bond breakage at the collision site, emitting atomic particles. As the collision cascade moves away from the collision site, collisions become less energetic and less fragmentation and bond breakage occurs resulting in the emission of molecular fragments [197]. Ionized species are accelerated by a fixed voltage through the flight tube to the mass analyser where in SIMS, positive and negative secondary ions (and neutral fragments) are separated and focused onto the detector. As all ions have the same kinetic energy at the beginning of the flight path, the differing mass of the ions results in differing velocities and them arriving at the mass analyser at different times allowing for their separation. Low mass ions arrive at the analyser faster than heavier ions. Equation 5.2 describes this effect and from the time it takes the ions to
travel the length of the flight tube, the mass of the ions can be calculated as shown in equation 5.3.

Equation 5.2: \[ E_K = \frac{1}{2} m V^2 \]

Where: \( E_K \) = kinetic energy, \( m \) = mass of ion, \( V \) = velocity of ion

Equation 5.3: \[ t - t_0 = L \left( \frac{m}{2E_K} \right)^{1/2} \]

Where: \( t \) = arrival time at detector, \( t_0 \) = start time from detector, \( L \) = length of flight path, \( m \) = mass of ion, \( E_K \) = kinetic energy

A reflectron helps compensate for energy and an angular dispersion that can occur during the emission process resulting in improved mass separation [197]. When analyzing polymers it is also necessary to provide surface charge stabilisation by means of a flood gun which floods the surface with electrons [206]. ToF-SIMS allows the chemical makeup of a sample to be mapped by focusing the primary ion beam to a narrow diameter and rastering it across the sample [199]. An immense number of peaks representing the full mass spectra of all ions can be obtained in a relatively short period of time. By evaluating the masses of the signals, peaks can often be identified from the molecular ion of the analyte, fragments of the molecular ion or ions of any other components that may be in the sample [197]. Specific ions or combinations can be selected and their distribution mapped using imaging software to establish how uniform their distribution is.
5.1.3.1. Time of Flight - Secondary Ion Mass Spectroscopy

Objective

In this study, ToF-SIMS was used to identify and map the distribution of the antimicrobial agents on the catheter surface and catheter cross sections through imaging and to track their profiles as the technique was used to penetrate the depths of the catheter. This was performed in order to assess how uniformly the drugs were distributed within the catheter which could affect drug release and bacterial colonisation. Catheters were examined both before and after 12 weeks in solution to elute the drugs from the antimicrobial catheter.

5.1.4. Water Contact Angle

Water Contact Angle (WCA) measurements have been a major approach used to investigate problems associated with solid - liquid interfaces [207]. The WCA is the angle at which the liquid interface meets a solid surface and is dependent on the surface chemistry and roughness. If water is placed on a solid surface the surface tension at the interfaces will cause the liquid to form a drop shape which has a defined contact angle (Figure 5.3). WCA measurements are extremely sensitive techniques that depend on the top layer of a surface and are therefore highly susceptible to surface contaminants and surface deformations. There are several ways to perform WCA measurements including static or sessile drop measurements where advancing and receding contact angles can be measured. If a contact angle is close to 0° the droplet will completely spread out on the surface, meaning the material is completely hydrophilic. A contact angle of less than 90° shows the liquid readily wets the material and the material is of a hydrophilic nature but less so than the above. A contact angle of >90° indicates that the liquid resists wetting and the
material is more hydrophobic. The degree to which a surface may be wet is a property that can influence bacterial attachment to surfaces.

![Diagram of contact angles](image)

**Figure 5.3:** The contact angle of a liquid at the interface of a solid. A contact angle (α) of >90° indicates low wettability and a hydrophobic surface. A contact angle of <90° indicates high wettability and a hydrophilic surface [208].

### 5.1.4.1. Water Contact Angle Objective

In this study, WCA measurements were performed on urinary catheters in the “as received” state from the manufacturer and on the antimicrobial incorporated catheters before and after 12 week soaking/drug elution to study if incorporation of the antimicrobial agents had any impact on the materials degree of hydrophobicity or hydrophilicity and therefore possible interaction within the urinary tract environment and bacterial colonisation.
5.2. METHODS

5.2.1. X-Ray Photoelectron Spectroscopy

6 mm silicone discs were impregnated with all three antimicrobial agents as described in section 2.2.4. Discs were also used without the incorporation of antimicrobial agents and served as controls. Half of both sets were exposed to sterile human urine (pH 6.8) for 1 hour in a 37°C incubator whilst the other half were left unprocessed. Care was taken to avoid possible contamination of surfaces. All four sets of discs were taken for XPS analysis to determine if a conditioning film had been deposited on the surface as indicated by the adsorption of proteins and other elements from the urine.

XPS spectra were recorded using a Kratos Axis Ultra spectrometer employing a monochromated Al K$_\alpha$ X-ray source (hv = 1486.6 eV), hybrid (magnetic/electrostatic) optics, hemispherical analyzer and a multi-channel plate and delay line detector with a collection angle of 30° and a take off angle of 90°. X-ray gun power was set to 100 W. All spectra were recorded using an aperture slot of 300 x 700 microns with a pass energy of 80 eV for survey scans and 20 eV for high-resolution core level scans. All XPS spectra were recorded using the Kratos VISION II software. Data files were translated to VAMAS format and processed using the CASA XPS™ software package (Version 2.3.2 and later).
5.2.2. Atomic Force Microscopy

1 cm catheter segments were impregnated with all three antimicrobial agents as described in section 2.2.4. Segments without antimicrobial agents served as controls. Half of each of the two sets of segments were placed in PBS for 12 weeks to simulate drug elution over the period of catheter use in the body. The buffer was replaced with fresh solution every week to prevent the build-up of antimicrobial agents. Small flat sections (approx 2 x 2 mm) of catheter segments were cut out and rinsed with ultra pure water and left to dry at room temperature. Sections were mounted onto metal stubs using superglue for analysis by AFM.

A MultiMode 8 AFM (Vecco Instruments) with a Nanoscope V controller operated in a PeakForce Quantitative NanoMechanical Property Mapping (QNM) mode in air was employed. A silicon nitride cantilever with a 0.1 N/m nominal spring constant and a silicon probe was adopted. The AFM was calibrated and two 5 x 5 µm surface area scans were performed on each catheter segment. The roughness of the surface was measured by the root mean square (Rq value) which is calculated by taking the centre line of a surface profile and calculating the root mean square deviation of a number of points on the profile from the centre line [199]. Rq values were obtained by taking the average of ten 1.3 x 1.3 µm areas from the two scans of each catheter segment. Values from control and antimicrobial catheter sections before and after soaking/drug elution were compared.
5.2.3. Time of Flight - Secondary Ion Mass Spectroscopy

1 cm catheter segments were impregnated with all three antimicrobial agents as described in section 2.2.4. Segments without antimicrobial agents served as controls. Half of each of the two sets of segments were placed in PBS for 12 weeks to simulate drug elution. The buffer was replaced with fresh solution every week to prevent the build-up of antimicrobial agents. All 1 cm segments were cut in half, rinsed with ultra pure water and left to dry at room temperature. Crude preparations of cross sections of the catheter segments were also cut to assess the distribution of the drugs through the thickness of the catheter tubing. Segments were also impregnated with rifampicin only, sparﬂoxacin only and triclosan only, without the 12 week drug elution for individual drug characterisation purposes.

ToF-SIMS analysis was performed using an ION-TOF IV (GmbH, Münster, Germany) instrument equipped with a Bi liquid metal ion gun (LMIG). Samples were mounted onto a sample holder using clamps and loaded into the ToF-SIMS vacuum chamber where pressure was maintained at \(10^{-8}\) to \(10^{-9}\) mbar. Images of the catheters outer surface were formed by rastering a pulsed Bi\(_{3}^+\) primary ion beam at a current of 1 pA. The pulsed Bi\(_{3}^+\) ion beam had a spot size of \(<2\ \mu\text{m}\) diameter and was rastered in a 256 x 256 pixel array. Charge compensation using an electron flood gun was employed due to the materials conductivity. Mass spectrum obtained from positive and negative ion profiling were calibrated using IonSpec Version 4.0 software supplied by the manufacturer. Ion intensities were normalized to the total ion count and spectrums generated from the surface of antimicrobial loaded catheters were compared to those without antimicrobial agents. A peak list was generated and those acting as markers for the material and drugs were identified. Ion image
Version 4.0 software was used to create images of the selected ions and drug
distribution analysed. Drug distribution across the thickness (cross-sections) of the
catheter tubing was assessed as above.

For depth profiling, the ToF-SIMS instrument was equipped with a 10 keV $C_{60}$ ion
source for sputtering as well as a 25 keV cluster Bi ion source for analysis, both set
at incident angles of 45°. An ‘interlaced’ mode was employed, utilizing a beam
target current of 200 pA for the $C_{60}^+$ sputter beam and a pulsed $Bi_3^+$ analysis beam
set to 0.25 pA. Sputtering and analysis beams were aligned and a sputter crater of
300 x 300 µm$^2$ etched with the central 100 x 100 µm$^2$ compartment analysed. Both
positive and negative spectra were calibrated as above and selected ions representing
markers of the drugs were reconstructed and exported into an excel spreadsheet to
allow the profiles of the agents to be studied.

5.2.4. Water Contact Angle

1 cm catheter segments were impregnated with all three antimicrobial agents as
described in Section 2.2.4. Segments without antimicrobial agents served as
controls. Half of each of the two sets of segments were placed in PBS for 12 weeks
to simulate drug elution. The buffer was replaced with fresh solution every week to
prevent the build-up of antimicrobial agents. Segments were rinsed with ultra-pure water and dried at room temperature for WCA measurements.

A KRÜSS Drop Size Analyzer (DSA) 100 with DSA software version 1.6 was used
to carry out WCA measurements. Each catheter segment was placed on the drop size
analyzer platform and 100 pL of ultra pure water was dispensed from a piezo dosing
unit onto the surface of the material. Four scans per sample were performed covering a range of 2 mm along the catheter length. A high speed camera was used to record the first second the spot of water touched the surface and a video image was taken. The droplet angle was determined by the DSA software package. A baseline was fitted to the water droplet circle and a tangent placed at the intersection of the liquid and solid. WCA were recorded and the average taken.

5.2.5. Statistics

Two tailed paired t-tests at the 95% confidence level were used to compare differences between control and antimicrobial catheter material.
5.3. RESULTS

5.3.1. X-Ray Photoelectron Spectroscopy

X-ray Photoelectron Spectroscopy was employed to determine if material exposed to urine for 1 hour resulted in the adsorption of urinary elements i.e. proteins and salts to the catheter surface. Wide survey scans were acquired to view all elements on catheter surfaces and high resolution scans performed on elements most commonly observed. The binding energy scale was referenced to the main C (1s) peak, attributable to hydrocarbon (CH). Relative atomic % were carried out using the peak area corrected with Kratos sensitivity factors supplied from the manufacturer and the average of three repeats reported as shown in Table 5.1.

Table 5.1: XPS elemental atomic relative % of control material (silicone without antimicrobial agents) and test material (silicone with antimicrobial agents) before and after exposure to urine for 1 hour to form a conditioning film layer. All tests were performed in triplicate and figures represent averages with standard deviations.

<table>
<thead>
<tr>
<th>Element</th>
<th>Control material minus conditioning film (± StDev)</th>
<th>Control material plus conditioning film (± StDev)</th>
<th>Antimicrobial material minus conditioning film (± StDev)</th>
<th>Antimicrobial material plus conditioning film (± StDev)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na 1s</td>
<td>0.0 ± 0</td>
<td>0.2 ± 0</td>
<td>0.1 ± 0</td>
<td>0.6 ± 0</td>
</tr>
<tr>
<td>O 1s</td>
<td>33.8 ± 1.2</td>
<td>29.6 ± 0.8</td>
<td>34.6 ± 0.7</td>
<td>25.8 ± 1.4</td>
</tr>
<tr>
<td>N 1s</td>
<td>0.2 ± 0.1</td>
<td>1.3 ± 0.2</td>
<td>1.2 ± 0.4</td>
<td>5.1 ± 1.2</td>
</tr>
<tr>
<td>Ca 2p</td>
<td>0.9 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>0.1 ± 0.2</td>
</tr>
<tr>
<td>C 1s</td>
<td>42.9 ± 1.8</td>
<td>47.6 ± 0.9</td>
<td>40.8 ± 0.9</td>
<td>54.8 ± 2.7</td>
</tr>
<tr>
<td>Cl 2p</td>
<td>0.4 ± 0.2</td>
<td>0.1 ± 0</td>
<td>0.3 ± 0</td>
<td>0.9 ± 0.3</td>
</tr>
<tr>
<td>Si 2p</td>
<td>19.2 ± 0.4</td>
<td>18.6 ± 0.4</td>
<td>18.2 ± 0.5</td>
<td>11.8 ± 2.4</td>
</tr>
<tr>
<td>Mg 2p</td>
<td>2.8 ± 0.3</td>
<td>2.0 ± 0.2</td>
<td>3.7 ± 0.2</td>
<td>0.8 ± 0.5</td>
</tr>
</tbody>
</table>

Deconvolution of C1s Peak

| C 1s aliphatic | 90.3 ± 1.2 | 85.7 ± 1.0 | 80.2 ± 3.3 | 68.3 ± 4.3 |
| C 1s C-OH     | 8.8 ± 0.8 | 11.7 ± 0.4 | 18.8 ± 2.8 | 23.8 ± 1.7 |
| C 1s COOH    | 0.9 ± 0.5 | 2.6 ± 0.7 | 1.0 ± 0.5 | 8.0 ± 2.7 |
Spectra from control and antimicrobial non-conditioned samples were compared to those with a conditioning film. Wide survey scans, shown in Appendix 4, did not reveal a big difference between conditioned and un-conditioned samples. High resolution scans however, highlight greater differences. Conditioned samples revealed changes in the elemental composition and relative % of elements. Elements of proteins e.g. nitrogen and carbon were found at increased levels on conditioned samples. The atomic % of oxygen however decreased following exposure to urine. Sodium was also found to increase upon conditioning whilst the atomic % of silicone decreased. Calcium and magnesium were found to be slightly higher on the un-conditioned samples. Figure 5.4 depicts the high resolution scans of the nitrogen and sodium peaks for both control and antimicrobial material before and after exposure to urine.
Chapter 5 - Surface Analysis

(A) N 1s

(B) N 1s

(C) N 1s

(D) N 1s

(E) Na 1s

(F) Na 1s
Figure 5.4: XPS high resolution scan of the Nitrogen (N) peak of control material (A) without a conditioning film (B) with a conditioning film. Nitrogen (N) peak of antimicrobial material (C) without a conditioning film (D) with a conditioning film. Sodium (Na) peak of control material (E) without a conditioning film (F) with a conditioning film. Sodium (Na) peak of antimicrobial material (G) without a conditioning film (H) with conditioning film.

The increase in carbon on conditioned samples also revealed the addition of a new functional group as depicted in Figure 5.5. The C (1s) peak on the non-conditioned samples was deconvoluted into two peaks. Conditioned samples showed evidence of a different chemical type of carbon atom. The peak with the lowest binding energy (282.0 eV) representing the reference peak of hydrocarbon (C-H) and the peak at 283.5 eV representing the addition of a single bond of either oxygen or nitrogen to the carbon (C-(O,N) are present on both conditioned and un-conditioned samples. For both conditioned control and antimicrobial material XPS revealed the addition of an extra peak at 285.5 eV, which is indicative of carbon making a double bond with oxygen (C═O).
Figure 5.5: High resolution scan of the deconvolution of the C 1s peak performed by XPS. Analysis is of control material (A) without a conditioning film (B) with a conditioning film and antimicrobial material (C) without a conditioning film (D) with a conditioning film. Forming a conditioning film on the control and antimicrobial material revealed the addition of an extra peak at 285.5 eV.
5.3.2. Atomic Force Microscopy

Atomic force microscopy was performed to study if elution of the antimicrobial agents from the catheter over a 12 week period had any impact on the surface roughness compared to material without antimicrobial agents. Two scans at different points on the catheters’ outer surface were taken and the roughness values (Rq values) determined by taking the average Rq of ten areas of size 1.3 x 1.3 µm on the scanned images. Table 5.2 shows the average Rq values of the catheter surfaces and Figure 5.6 shows the topographical images of control and test catheter segments before and after soaking/drug elution.

Table 5.2: Roughness (Rq) values as determined by AFM for control and antimicrobial catheter segments before (p = 0.26) and after (p = <0.05) 12 week soaking/drug elution in buffered solution. Values reported represent the average of 2 scans upon which 10 areas were selected for average Rq determination with standard deviations.

<table>
<thead>
<tr>
<th>Catheter Treatment</th>
<th>Rq Value (nm) (± St Dev)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control catheter material prior to drug elution</td>
<td>12.53 ± 1.79</td>
</tr>
<tr>
<td>Antimicrobial catheter material prior to drug elution</td>
<td>13.14 ± 1.40</td>
</tr>
<tr>
<td>Control catheter material post drug elution</td>
<td>9.57 ± 1.35</td>
</tr>
<tr>
<td>Antimicrobial catheter material post drug elution</td>
<td>22.72 ± 4.33</td>
</tr>
</tbody>
</table>

The impregnation of rifampicin, sparfloxacin and triclosan into the catheter material revealed no significant difference (p = 0.26) in surface roughness compared to the material without antimicrobial agents. Elution of the antimicrobial agents revealed a significant difference (p = <0.05) in the roughness value between the antimicrobial and control material. Catheter material containing the antimicrobial agents following elution of the drugs were found to have a significantly higher Rq value.
Figure 5.6: AFM roughness analysis images of catheter segments. (A) control catheter material before soaking compared to (B) antimicrobial catheter material before drug elution (soaking) (p = 0.26) and, (C) control catheter material after 12 week soaking compared to (D) antimicrobial catheter material after 12 week drug elution (soaking) (p = <0.05). Antimicrobial catheter segments following drug elution (Image D) show a greater degree of surface roughness.
5.3.3. Time of Flight - Secondary Ion Mass Spectroscopy

Time of flight - secondary ion mass spectroscopy was used to identify the antimicrobial agents within the polymer and analyse their distribution on the surface through imaging. A large number of peaks were generated when scanning the surface of the control and antimicrobial catheter material. To identify markers of the three drugs, positive and negative ion control material spectra were compared with antimicrobial material spectra. The chemical formulae of the antimicrobial agents are given below:

- Rifampicin \( \text{C}_{43}\text{H}_{58}\text{N}_{4}\text{O}_{12} \)
- Sparfloxacin \( \text{C}_{19}\text{H}_{22}\text{F}_{2}\text{N}_{4}\text{O}_{3} \)
- Triclosan \( \text{C}_{12}\text{H}_{7}\text{Cl}_{3}\text{O}_{2} \)

Mass spectra may display the whole molecular ion peak, but this does not always appear and can be weak. Peaks corresponding to fragments of the molecular ion may also represent markers of the drugs or isotope peaks. Alternatively the molecular formula of each drug may reveal elements that enable the agents to be traced. Mass spectra obtained from positive and negative secondary ions were viewed and the negative spectra revealed peaks that could be identified to the drugs. Figure 5.7 shows a typical mass spectrum of control and antimicrobial material.
Figure 5.7: Negative ion spectrum for (Top Image) control catheter material (Lower Image) antimicrobial catheter material (before soaking/drug elution). CN = carbon-nitrogen, $^{13}\text{CN}$ = carbon-nitrogen isotope, Cl = chlorine, $^{37}\text{Cl}$ = chlorine isotope, F = fluorine, Si = silicone, SiH = silicone-hydrogen. The antimicrobial material shows the presence of F, CN and Cl ions.
The negative ion spectra revealed that flourine ions (F⁻), chlorine ions (Cl⁻), its isotope (Cl³⁷) and carbon-nitrogen (CN⁻) could be acting as markers for the drugs. It is likely that as the molecular formula of sparfloxacin contains F⁻, this element could be acting as its marker and as triclosan contains Cl⁻ in its structure, this and its isotope are likely to be representative of the drug. CN⁻ can be found in both rifampicin and sparfloxacin but not triclosan as it does not contain nitrogen. To determine which drug CN⁻ acted as a marker for, catheter material was impregnated with individual drugs. Spectra obtained from rifampicin containing catheter segments showed no evidence of any distinguishing elements. Neither CN⁻, the molecular ion or any fragment of rifampicin were at detectable levels. This drug was therefore unable to be characterised. CN⁻ was therefore also a maker for sparfloxacin as revealed from spectra obtained when material was impregnated with sparfloxacin only. The control material was also processed in the same manner as the antimicrobial catheter via exposure to chloroform (CHCl₃) and the spectra shown in Figure 5.7 revealed that there was little Cl⁻ contribution from the CHCl₃ to significantly impact on the Cl⁻ peak from triclosan. Comparison of the spectra (data not shown) for control material exposed to chloroform against control material in its “as received” state was also used to infer that there was no residual CHCl₃ in the silicone. Gas chromatography of the samples would be further required to confirm this. It was therefore only possible to confirm markers for two out of the three drugs. It can be surmised that F⁻ and CN⁻ are good indicators for sparfloxacin and Cl⁻ (and isotope) for triclosan.

Figure 5.8 shows images of selected elements F, Cl, CN⁻, Si (silicone), SiO₂ (silicone dioxide) and the total ions obtained from surface scans of the control and antimicrobial catheters.
Figure 5.8: Negative ion ToF-SIMS images of the surface of (A) control catheter material and (B) antimicrobial catheter material before soaking/drug elution and (C) control catheter material and (D) antimicrobial catheter material after 12 week soaking/drug elution. Chlorine (Cl\(^{-}\)) represents triclosan, fluorine (F\(^{-}\)) and CN\(^{-}\) represent sparfloxacin whilst rifampicin could not be traced. The presence of the agents on the antimicrobial catheter surface is depicted by the more intense shade appearing relatively evenly distributed although a localization of drug can be seen (Image B) which diffuse away following drug elution (Image D). Cl\(^{-}\) on Image C is likely to be due the buffer used to soak the catheter in. The silicone (Si) and silicone dioxide (SiO\(_2\)) elements form part of the material and are shown to be uniformly disturbed on all images. Total ion images are also given.
The images in Figure 5.8 allowed for the distribution of drugs on the outer catheter surface to be assessed for uniformity. Due to the swelling nature of the polymer upon immersion into the chloroform, it was expected that the drugs would be distributed the same on the outer surface as on the luminal surface. Image A of the control material, shows Si and SiO$_2$ to be representative of the polymer. Control material exposed to 12 weeks in buffer solution in the same way antimicrobial-containing catheter segments were, revealed traces of Cl$^-$ (Image C). This can however be attributed to the components within the buffer adsorbing to the surface. Images of Si and SiO$_2$ remain similar following soaking. The antimicrobial catheter prior to drug elution (Image B) showed a fairly even distribution of sparfloxacin on the catheter surface indicated by the intensity of F$^-$ and CN$^-$ across the image. Cl$^-$ as a marker for triclosan was also fairly evenly distributed across the catheter surface. Image B also revealed the presence of aggregates of drugs formed on the catheter surface. Following drug elution the Cl$^-$ intensity remained as triclosan continued to diffuse to the surface with some contribution from the buffer whereas the sparfloxacin markers, F$^-$ and CN$^-$, appeared less intense.

Depth profiling was used to trace the distribution of the chemical species representing sparfloxacin and triclosan as a function of depth from the surface. Figures 5.9 and 5.10 follow the profiles of the antimicrobial agents as they were tracked through the polymer.
Figure 5.9: Depth profiling performed by ToF-SIMS of (A) control catheter material and (B) antimicrobial catheter material before soaking/drug elution. Fluorine (F) and carbon-nitrogen (CN) represent sparfloxacin, chlorine (Cl) triclosan and silicone (Si) the catheter material.
Figure 5.10: Depth profiling performed by ToF-SIMS of (A) control catheter material and (B) antimicrobial catheter material after soaking/drug elution. Fluorine (F) and carbon-nitrogen (CN) represent sparfloxacin, chlorine (Cl) triclosan and silicone (Si) the catheter material.
F, Cl⁻ and CN⁻ intensities are negligible in the control material both before and after 12 weeks soaking. Si intensities were high at the surface and decreased as the material was penetrated. It was expected that Si and SiO₂ may have increased or remained the same throughout the depth profiling, but this may have been due to the degradation effects from the C60 beam. The control material when soaked showed a lower intensity of silicone. Image B displaying the profile for the antimicrobial material shows Cl⁻, F⁻ and CN⁻ intensities to peak at the surface and then decline through the bulk. Cl⁻ therefore triclosan intensities are greater than for the sparfloxacin markers F⁻ and CN⁻. Cl⁻ intensities remain constant deep into the polymer whereas F⁻ and CN⁻ display a slight decline the deeper the scan continues. It is evident that upon immersing the antimicrobial material into an aqueous environment for 12 weeks (Image D), the intensities of the elements representative of sparfloxacin and triclosan are reduced. There is a peak of Cl⁻ at the surface which is followed by a rapid decline to steady state conditions whilst the intensity of F⁻ and CN⁻ (sparfloxacin) are less than the Cl⁻ (triclosan) and similar to the Si intensity.

Images from cross sections of catheters are given in Figure 5.11. Images were created by scanning the inner to the centre of the tubing and the centre to the outer of the tubing and viewing both to assess the uniformity of drug distribution through the thickness of the catheter. Figure 5.11 depicts the scans taken from the inner catheter wall to the centre of the section. Diagrams show the location of the scans on the cross sectional areas. Combined viewing of the inner scans and outer scans (data not shown) revealed that sparfloxacin and triclosan penetrated the total thickness of the catheter wall.
Figure 5.11: Images taken from the inner catheter wall to the centre of the cross sectional area along with diagrams depicting the scan location of the (A) control catheter material and (B) antimicrobial catheter material before soaking/drug elution (C) control catheter material and (D) antimicrobial catheter material after soaking/drug elution (E) rifampicin only (F) triclosan only (G) sparfloxacin only catheter material before drug elution. Images reveal the antimicrobial agents penetrate the thickness of the catheter wall.
5.3.4. Water Contact Angle

Water contact angle measurements were taken to discover if impregnating the catheter material with antimicrobial agents affected the degree of hydrophobicity/hydrophilicity displayed by the material. Table 5.3 shows the average WCA measurements of control and antimicrobial catheter material prior to and post soaking/drug elution.

Table 5.3: WCA measurements of control and antimicrobial catheter material before (p = 0.015) and after soaking/drug elution (p = >0.05). All tests are the average of 4 replicates with standard deviation and coefficient of variation.

<table>
<thead>
<tr>
<th></th>
<th>WCA (°) of control catheter material before soaking</th>
<th>WCA (°) of antimicrobial catheter material before drug elution</th>
<th>WCA (°) of control catheter material after soaking</th>
<th>WCA (°) of antimicrobial catheter material after drug elution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Average</strong></td>
<td>90.68</td>
<td>79.95</td>
<td>84.73</td>
<td>78.33</td>
</tr>
<tr>
<td><strong>St Dev</strong></td>
<td>0.85</td>
<td>3.88</td>
<td>3.73</td>
<td>1.55</td>
</tr>
<tr>
<td><strong>CV</strong></td>
<td>0.01</td>
<td>0.05</td>
<td>0.04</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Results showed that there was a statistically significant difference (p = 0.015) in the WCA measurement when antimicrobial agents were added to the silicone catheter. The WCA decreased making the antimicrobial catheter slightly more hydrophilic. No significant difference (p = >0.05) was seen between the control and antimicrobial catheter segments following exposure to an aqueous environment or between catheters of the same type (p = >0.05) that were compared before and after soaking/drug elution.
5.4. DISCUSSION

5.4.1. X-Ray Photoelectron Spectroscopy

The implantation of any medical device into the body is followed by the formation of a conditioning film onto its surface. Conditioning films formed on urinary catheters are largely composed of proteins, electrolyte components and other unidentified molecules [10] and have the ability to change the physico-chemical properties of a material's surface which can affect interactions between bacteria and the surface, although this is dependent on the substrate material, the composition of urine which is influenced by a patient’s individual condition and on the bacterial strain. Confirmation that exposing the material to urine for 1 hour was sufficient time for a conditioning film to form was necessary to ensure that adherence assays (tk100) were carried out in a way that is representative of the process within the body. A variety of analytical techniques have been used in particular to study protein adsorption to biomaterial surfaces. Examples include ultra violet spectroscopy, fluorescent labelling, quartz crystal microbalances (QCM), AFM, surface plasmon resonance (SPR), XPS as well as molecular techniques [197]. Santin et al (1999) [166] characterised the protein composition of the conditioning film deposited onto the surface of ureteral stents during in-vivo implantation by SDS-PAGE and Western blot. Results showed the presence of albumin, Tamm-Horsfall Protein and α1-microglobulin adsorption.

In this study, XPS was used to confirm that elements of proteins and other components from the urine adsorbed to the catheter material as an indication of the formation of a conditioning film. The technique was not used to quantify the levels
of protein or for the differentiation between proteins. Proteins are mostly composed of carbon, oxygen and nitrogen and lower levels of other elements such as phosphorus. Tieszer et al (1998) [10] observed through XPS studies the conditioning film elements associated with ureteral stent removal from patients. Most notable was the detection of calcium and phosphorus albeit in small amounts, four different chemical types of carbon atom, an increase in oxygen and nitrogen and a decrease in silicone. Similar observations were revealed in this study as shown in Table 5.1. An increase in carbon and nitrogen was seen on the conditioned samples indicating that some degree of protein adsorption had taken place. Sodium deposits also rose on conditioned samples possibly due to the presence of salts within the urine. A decrease in the silicone concentration resulting from the deposition of a surface conditioning film was found which masked the silicone - rich surface. Calcium and magnesium levels were less on the conditioned samples. Wollin et al (1998) [196] report on calcium, magnesium and phosphorus, elements of encrustation observed upon removal of ureteral stents following >11 days implantation, so it is not surprising to expect calcium and magnesium to adsorb at such a rate after a much shorter exposure time to urine. XPS also revealed on conditioned control and antimicrobial material the addition of an extra peak at 285.5 eV, which is indicative of carbon making a double bond with oxygen (C═O). Reid et al (1994) [170] also revealed the addition of carbonyl groups that adsorbed to the ureteral stents following 24 hours incubation in human urine. The lowest binding energy of the C1s peak C-C or C-H are usually found as part of the polymer or as a surface contaminant from the air [170]. C═O and/or COOH additional groups added after conditioning show the conversion of C-O groups to more acidic or oxygenated groups due to the adsorption of a conditioning film [10, 170, 196]. The increase in
carbon, nitrogen and sodium, the decrease of silicone and addition of the carbon group suggest that a conditioning film had been formed on the surface of the catheter material. The conditioning effect agrees with Lappin-Scott and Costerton [45] who say that adsorption of molecules onto surfaces occurs rapidly. One hour exposure to urine is therefore sufficient time for a conditioning film layer to form and confirms that adherence assays were performed in a manner that reflected the conditioning film process as catheters are inserted into the body.

5.4.2. Atomic Force Microscopy

An important component of catheter surfaces is their degree of roughness/smoothness as this can affect the ease of catheter insertion/removal and potential for bacterial colonisation. Although appearing smooth, urinary catheters can show a variety of topographical features, ranging from smooth, to undulated to rough [170]. Surface roughness can be a result of the manufacturing process and can also be induced through long-term use in a patient. In this study the effect of impregnating antimicrobial agents into the catheter had on the degree of surface roughness was observed using AFM and the impact drug elution over 12 weeks had on surface roughness values was also monitored. Results showed that there was no significant difference in Rq values (p = 0.26) between material with and without antimicrobial agents prior to drug elution. This suggests the drugs on the surface are the same size as the polymer making the surface topography equally smooth. Control material appeared smoother following exposure to an aqueous environment (Rq = 9.57 nm) where as the antimicrobial materials roughness value significantly increased (Rq = 22.72 nm) (p = <0.05). This may imply that the drugs aggregate at
the surface and as they diffuse into the surrounding medium their release imparts a rougher surface.

Studies have shown that irregularities of polymeric surfaces can promote bacterial adhesion and biofilm deposition [204, 205]. It is thought that this may happen since a rough surface has a greater surface area and the depressions in the surface provide more favourable sites for colonisation [52] with the greatest initial accumulation of bacteria being in the bottoms of the roughest parts of the surface where they are also protected from shear forces. It is recognised that the size of the irregularities is important for bacterial colonisation, not just the degree of roughness, but without further studies it is difficult to establish if the 13.15 nm increase in the roughness value following elution of the antimicrobial agents would have had a direct impact on bacterial colonisation.

Surface roughness is also important to consider in ensuring maximal ease of catheter insertion and removal. Urinary catheters with rougher surfaces can cause more trauma, inflammation and discomfort to the patient upon insertion and removal than those of a smoother nature. Jones et al (2001) [209] studied the relationship between the ease of catheter removal and surface roughness and found the relationship to be significant in which catheter lubricity increased as surface roughness decreased. As no difference in surface roughness between control and antimicrobial catheters was seen, this suggests that insertion of the antimicrobial catheter would be no different from the conventional Foley catheter. It is also not envisaged that the increase in roughness following drug elution would impinged on catheter removal as the increase was small.
5.4.3. Time of Flight - Secondary Ion Mass Spectroscopy

Exploration of the surface and bulk properties of antimicrobial polymers is important clinically as the distribution of the drug can influence the extent and rate of drug release. ToF-SIMS was able to provide qualitative information on the uniformity of drug distribution. Cl⁻ ions and F⁻ / CN⁻ ions served as markers for triclosan and sparfloxacin respectively but rifampicin did not reveal any secondary ions that enabled for its characterisation so was therefore unable to be traced. Images of the antimicrobial catheter surface prior to drug elution (Figure 5.8) revealed that sparfloxacin and triclosan were relatively evenly distributed but some aggregation of the drugs was seen (Image B) which upon three-dimensional viewing suggests that these penetrate part-way into the polymer. It is possible that the localization of the drugs on the surface may have added to the burst effect first seen when the catheter is exposed to an aqueous environment and that following 12 weeks of drug elution (Image D) the aggregates diffuse out. This burst effect may aid in reducing bacterial colonisation at the time of catheter insertion.

The depth profile graphs (Figures 5.9 and 5.10) show triclosan and sparfloxacin intensities to decrease through the bulk, suggesting that the drugs diffuse from the bulk of the polymer to the inner and outer surfaces where drug intensities are at their highest. It is important to acknowledge that the C60 beam used in depth profiling can leave deposits of carbon on the surface which may mask the underlying material. Freezing the sample can act to reduce the carbonisation effect, but in this instance this was felt unnecessary as Cl⁻, F⁻ and CN⁻ ions were clearly detectable. Elution of the drugs over the 12 week period showed lower intensities of sparfloxacin and triclosan but there was a greater intensity of triclosan compared to sparfloxacin. This
finding correlates well to the results obtained from the SPTT in which zone sizes of susceptible organisms exposed to triclosan were greater and more persistent than for sparfloxacin.

Viewing the cross-sectional areas of the catheters (Figure 5.11) revealed that the antimicrobial agents are distributed across the thickness of the catheter. Inspected together with the graphical information (Figures 5.9 and 5.10) of the depth profile analysis suggests drugs diffuse constantly to give higher intensities at the inner and outer surfaces which lasts to in excess of 12 weeks. To obtain improved images of the cross sections it may have been necessary to generate more accurate and thinner preparations of the sections using a microtome which could have been set in resin. This may have enabled the whole width of the cross sections to be analysed without creating edge effects as opposed to the inner to middle and middle to outer parts being examined in two halves.

5.4.4. Water Contact Angle

The WCA measurement was used to determine the impact impregnating antimicrobial agents into the catheter material had on the polymer’s degree of hydrophobicity/hydrophilicity. A significant decrease (p = 0.015) in the CA was observed when plain silicone catheters were impregnated with antimicrobial agents, indicating the change to a slightly more hydrophilic biomaterial surface. As catheter sections were exposed to 12 weeks in an aqueous environment, control and antimicrobial catheters showed no significant difference in CA measurements. Busscher et al (1984) [210], report on how surface roughening tended to increase the CA if the CA on the surface was >86° but it decreased the CA if the surface was
<60°, whilst in between 60 - 84° roughening had no influence on the CA measured. In contrast Chow et al (1998) [211] showed that the roughening of polymer surfaces reduced the CA and McGovern et al (1997) [212], found that the CA also decreased when polyurethane urinary catheters were incubated in urine and/or PBS for 24 hours. It is possible that the nature of the polymer is important for CA measurements. In this study the increase in surface roughness as the drugs eluted showed a decrease in CA although this was not significantly different (p = 0.556) from the antimicrobial material before drug elution.

It has been suggested that an increase in polymer hydrophobicity can act to promote bacterial adherence whereas more hydrophilic materials result in less bacterial adherence [77]. The more hydrophilic nature of the antimicrobial material would therefore appear to be positive and although CA measurements of surfaces appears to be a useful method of assessing changes arising in biomaterial surfaces in contact with fluids, further studies are required to determine direct influence CA over antimicrobial ability may have in respect of the urinary catheters response to bacterial colonisation.
5.4.5. Summary

In conclusion, as complications such as bacterial colonisation and encrustation associated with urinary catheters occur at the surface that interfaces with the urinary tract environment, analysis of the surface of newly developed materials is important to ensure that the characteristics of the new materials compare well to existing ones or are improved upon so to not adversely impact on bacterial colonisation. XPS has confirmed an hour exposure to urine is sufficient for a urinary conditioning film to be formed on the surface which is important in the bacterial attachment process. AFM used to compare the roughness of the antimicrobial catheter surface to the control catheter surface showed there was no significant difference but following soaking/drug release for 12 weeks the roughness value of the antimicrobial catheter surface increased, possibly due to elution of the drugs imparting a rougher polymer surface. It is however thought that this would not be to a degree that would be likely to promote bacterial adherence (although further testing would be required) or have a negative impact on the ease in which the catheter is inserted or removed. CA measurements revealed the antimicrobial material to be more hydrophilic, upon which reports suggest bacteria adhere least to more hydrophilic materials [77] and that upon drug elution no difference in CA was seen compared to control material. ToF-SIMS revealed that the impregnation process used to incorporate the antimicrobial agents into the catheter distributes them (triclosan and sparfloxacin) relatively evenly on the catheter surface with some drug aggregation which could aid in the prevention of infection during catheter insertion due to the possible added burst effect. Drugs were shown to penetrate the whole cross section of the catheter with drugs diffusing from the bulk to the inner and outer surfaces where drug intensities are highest and where they become released into the surrounding
environment. The homogeneous nature following the diffusion of the drug aggregates on the surface suggests that drugs release in a uniform manner which is important for the killing of bacteria over time.
CHAPTER 6

MECHANICAL PROPERTY TESTING
6.0. MECHANICAL PROPERTY TESTING

6.1 INTRODUCTION

When making a urinary catheter antimicrobial, questions must be asked as to whether it has any effect on mechanical performance. As with all catheters, the correct physical and mechanical properties are essential if a product is to be successful in clinical use. Failure of mechanical performance could seriously endanger patients. Specifications, to which catheter devices and materials must conform, exist in the form of British (BS) and American Standard Test Methods (ASTM). Performance specifications establish performance requirements and describe associated tests that will ensure the safety and effectiveness of medical devices.

There is currently no standard for testing the mechanical properties of long-term indwelling urinary catheters (>30 days). This leaves long-term catheters open to separate standard development. The ASTM F623 99 (Reapproved 2006) [213], standard performance specification for Foley catheters and BS EN 1616: 1997 specifications [214] relate to single use, balloon retention urethral catheters but are for short-term use. They relate to catheters made from various materials such as silicone and latex, of sizes 12 to 26 French either in a sterile state for single use or non sterile state for sterilisation. Catheters for short-term use whose surfaces have been modified to reduce microbial colonisation may also be tested using these specifications. The FDA: Guidance on Premarket Notification [510(K)] Submission for Short-Term and Long-Term Intravascular Catheters specification [215] issues guidance on mechanical testing of catheters intended for long-term use (30 days or
more). However, the FDA has not currently classified devices such as urinary catheters intended for use for more than a 30 day placement. These devices are ‘unclassified’. An interpretation of the above specifications was however used as a guide upon which tests were established to perform on urinary catheters for long-term use.

According to the BS EN 1616:1997 [214], the shaft, tip, balloon and eyeholes of urinary catheters should have a smooth finish. By ensuring this, patient comfort, and mechanical trauma upon insertion and removal are kept to a minimum [12]. Flexibility of the catheter shaft is also important for manoeuvring through the urethra and a pliable catheter tip is necessary to reduce the occurrence of urethral perforation during insertion. The material must also be of a suitable strength and sufficiently resilient throughout its use to be capable of performing its primary function. Whilst a catheter is in situ and upon its removal it can be subject to significant tensile forces. The catheter must be able to withstand the effects of the forces encountered which must not result in damage, detachment of the shaft from the balloon or the separation of the catheter into various parts [12]. The catheter balloon should be strong enough to resist rupture and leakage but allow for ease of inflation and deflation, making elastic recovery an important parameter [12]. Despite these stipulations there are very few data in the literature on the physical and mechanical properties of urinary catheters prior to or following clinical use.
Mechanical property parameters typically measured for vascular catheters are listed below [215]:

- Tensile strength of catheter body
- Tensile strength of catheter body to hub attachment
- Catheter stiffness
- Catheter tip (distal) attachment strength
- Catheter elongation
- Leakage at hub
- Catheter burst pressure (positive internal pressure)
- Catheter collapse (negative internal pressure)
- Catheter flexural fatigue tolerance

Mechanical properties of materials and their components are commonly assessed using Instrons. Typical tensile testing involves clamping a sample with a fixed cross-sectional area to the Instron which serves to stretch the material in a controlled manner with increasing force until the sample becomes deformed (elongated) or breaks. For all testing applications, correct gripping of the device is essential to obtain accurate measurements. Avoidance of slippage and premature breakage of materials due to the jaws used to clamp material must be avoided. The use of video extensometers that are capable of performing stress/strain measurements have helped to gather more accurate measurements by capturing continuous images of markers on the specimen during testing.
6.1.1. Objectives

The incorporation of antimicrobial agents as a means of preventing bacterial colonisation on catheter surfaces warrants investigation into the possible effects that this may have on physical and mechanical parameters. This is especially relevant if catheters are modified from proven types. From a biocompatibility and patient safety prospective it is important that the inclusion of antimicrobial agents do not serve to adversely affect the mechanical properties of the catheters and balloons. This chapter assesses the mechanical performance of the catheter shaft and balloon both before and after 12 week simulation in a physiological environment designed to reflect the period of placement within the body and compares it to standard Foley catheters without anti-infictive properties. It was expected that due to the small percentage of antimicrobial agents incorporated into the catheter that this would not adversely affect the mechanical performance of the catheter. The tests described in Section 6.2 are those that are considered most relevant for long-term antimicrobial urinary catheters adapted from the ASTM F623 99 (Reapproved 2006) standard, BS EN 1616: 1997 specification and the FDA: Guidance on Premarket Notification [510(K)] Submission for Short-Term and Long-Term Intravascular Catheters specification. Tests performed are those which were able to be undertaken within the laboratory’s capability.
6.2. METHODS

6.2.1. Catheter Balloon Testing [213, 214]

6.2.1.1. Surface Finish

After the incorporation of antimicrobial agents into the catheter material as described in Section 2.2.4, catheters were viewed by normal vision and at X2.5 magnification. The surfaces of the catheter shaft, tip, balloon and eyes were examined for extraneous material. To pass the test, antimicrobial catheters were compared to urinary catheters without antimicrobial agents (controls) for freedom from extraneous material.

6.2.1.2. Strength

Antimicrobial and control catheter balloon strength tests were carried out after immersion of the inflated balloons in artificial urine (Appendix 5) for 30 days at 37°C. Catheters were then suspended from a suspension device by passing a pin of 50 - 75% of the diameter of the eyelet through the drainage eye. A 1 kg weight was attached to the drainage funnel and held for 1 minute. All unions of the catheter were examined for detachment or failure of bonds and any signs of the eyes splitting.

6.2.1.3. Connector security

This test was designed to simulate a urinary collection bag attached to the drainage funnel upon which the drainage funnel should not become detached from the test
connector. A 1 kg weight was attached to the drainage funnel of the antimicrobial and control catheters and held for 1 minute. If the test connector parted from the drainage funnel, this was deemed a failure.

6.2.1.4. Balloon Integrity

To test balloon integrity, antimicrobial and control balloons were inflated with 10 mL distilled water and submerged into artificial urine at 37°C for 30 days. Catheter balloons that ruptured were deemed to have failed the test.

6.2.1.5. Balloon security

Antimicrobial and control catheter balloons were immersed in artificial urine at 37°C for 30 days. The catheter tip and balloon were then placed through a funnel and the balloon re-inflated to 10 mL and allowed to rest in the funnel countersink. A 1 kg weight was attached to the catheter drainage funnel and allowed to hang for 1 minute. A failure was incurred if upon examination the balloon leaked or eyeholes were occluded.

6.2.1.6. Inflated Balloon Response to Pullout

This test assesses the catheter’s response to forces occurring during use. A funnel apparatus with a 28 French lumen was used to represent the bladder outlet and urethra and the proximal end of the antimicrobial or control catheter passed through the funnel inlet and the balloon inflated with 10 mL distilled water. The balloon portion was pulled back until it rested into the V shape of the funnel and the distal
end of the catheter hung downwards from the funnel barrel. For the static load test a 1 kg weight was hung from the distal end of the catheter for 2 minutes and the balloon observed. For the impact load test, the weight was lifted 60 cm from its hanging position and released. The balloon was observed for its position in the funnel after the weight had been dropped. For both tests, the passage of the balloon through the funnel or the deflation or rupture of the balloon resulted in a failure.

6.2.1.7. Balloon Volume Maintenance

This test was performed to test the ability of the balloons to retain their injected balloon volume as a means of determining the functional integrity of the inflation system. The antimicrobial and control catheter balloons were inflated with 10 mL methylene blue crystal solution (Sigma-Aldrich) (1 g in 2000 cm³ water). The balloon was placed on a surface that allowed for any leakages to be easily detected whilst protected from light for 30 days. A failure of the balloon to retain the solution was indicated when a discolouration or leakage of the methylene blue was observed on the surface beneath the balloon. Evaporation was accounted for by re-inflating the balloon to the specified volume.

6.2.1.8. Deflation Reliability

The test was designed to detect the failure of the balloon or the shaft lumen to deflate correctly in a simulated withdrawal. Antimicrobial and control catheter balloons were filled with 10 mL distilled water and placed into artificial urine for 30 days at 37°C. Balloons were then deflated using a syringe and the resultant deflated balloon
shape/size measured. To pass the test it should pass through a French gauge hole no more than four French sizes larger than the labelled French size.

All tests were carried out in triplicate.

6.2.2. Catheter Tensile Testing

Urinary catheters were used in the “as received” state from the manufacturers (controls) and following impregnation with the antimicrobial agents as described in Section 2.2.4. Half of the control and antimicrobial catheters were placed in simulated urine (Appendix 5) which was replaced weekly for up to 12 weeks to mimic drug elution in the body, whilst the other half remained in an unchanged state. Catheter balloons and junctions were not subjected to testing.

Mechanical performance of catheter shafts was assessed using an Instron 5985 (Imetrum Limited, Bristol, UK) with a 5 kN loadcell connected to a video extensometer. Data were recorded using Bluehill 2 software. Various clamping methods were investigated and a specialised configuration was devised to connect the catheter tubing to the Instron as is shown in Figure 6.1. This was required to avoid catheter slippage and breakage at the point of connection and to guarantee an even distribution of stress/strain along the length of the tested catheter. Catheters were clamped to expose a 5 cm length along the mid-section of the catheter and coated metal targets were applied 3 cm apart to allow for tracking by the video extensometer. A force was applied at a rate of 100 mm/min as the apparatus recorded the stress. The force applied was continued until either the apparatus reached its maximum length limit or until catheter failure (breakage) occurred. All
tests were conducted at room temperature and in all cases three replicates of each analysis were performed. From the resultant relationship between the stress and strain, the load (N), ultimate tensile strength (MPa), elongation at break (mm) and modulus (MPa) determined at 280 - 320% elongation were calculated.

Figure 6.1: Catheter set up on the Instron. (A) prior to tensile testing (clamping mechanisms consisted of clamping the catheter in an s-shaped configuration ensuring that the clamped portion was under no more excessive stress than the tested portion of the catheter) (B) during tensile testing.

6.2.3. Statistics

Two tailed paired t-tests at the 95% confidence level were used to compare differences between control and antimicrobial catheter material.
6.3. RESULTS

6.3.1. Catheter Balloon Testing

Table 6.1: Control and antimicrobial catheter balloon testing results.

<table>
<thead>
<tr>
<th>Test Requirement</th>
<th>Control Catheter</th>
<th>Antimicrobial Catheter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface finish</td>
<td>Freedom from extraneous material Pass</td>
<td>Freedom from extraneous material Pass</td>
</tr>
<tr>
<td>Strength</td>
<td>All unions intact Pass</td>
<td>All unions intact Pass</td>
</tr>
<tr>
<td>Connector security</td>
<td>Drainage funnel and connections intact Pass</td>
<td>Drainage funnel and connections intact Pass</td>
</tr>
<tr>
<td>Balloon integrity</td>
<td>No rupture or leakage Pass</td>
<td>No rupture or leakage Pass</td>
</tr>
<tr>
<td>Balloon security</td>
<td>No balloon leakage or eyehole occlusion Pass</td>
<td>No balloon leakage or eyehole occlusion Pass</td>
</tr>
<tr>
<td>Inflated balloon response to pullout</td>
<td>No deflation or rupture of balloon Pass</td>
<td>No deflation or rupture of balloon Pass</td>
</tr>
<tr>
<td>Balloon volume maintenance</td>
<td>Retained solution/no leakage Pass</td>
<td>Retained solution/no leakage Pass</td>
</tr>
<tr>
<td>Deflation reliability</td>
<td>Reliable deflation, passed through &lt; 4 labelled French size hole Pass</td>
<td>Reliable deflation, passed through &lt; 4 labelled French size hole Pass</td>
</tr>
</tbody>
</table>
Table 6.1 shows that the antimicrobial balloon results were identical to the control catheter balloons. The drainage funnel and all unions remained intact and no occlusions of eyeholes were seen when challenged with 1 kg weights. The antimicrobial balloon remained secure and maintained its integrity throughout with no rupture or leakage occurring at any point. Deflation of the antimicrobial balloon occurred in a reliable fashion and was within 4 French units from the original size (Figure 6.2). Overall no significant differences were displayed between the control and antimicrobial balloons.

![Figure 6.2](image-url): Control and antimicrobial catheter balloons (A) inflated (B) deflated after 30 days.
6.3.2. Catheter Tensile Testing

Figure 6.3 shows a typical stress-strain curve for control silicone urinary catheters and Table 6.2 the parameters measured. The graph displays the force applied to the catheter continuously increasing up until the apparatus reached its maximum length limit. At the stage where the catheter was continually stretched it appeared to become deformed and as this coincided with the limits of the Instron this point was taken as the tensile strength of the material. The stress-strain curve where strain is any kind of deformation, including elongation, can be used to determine the modulus which relates to a material’s ability to resist deformation. The slope of the plot (taken at 280 - 320% strain) relates to the modulus value. High modulus values can be interpreted as a material which resists deformation and a modulus of a low value suggests a material which is easily deformed.

![Stress-strain curve](image)

**Figure 6.3:** Typical stress-strain curve (depicting three control catheters following 12 weeks soaking in an aqueous environment).
Table 6.2: Load, tensile stress and modulus values of control and antimicrobial catheter shafts before soaking/drug elution and after 12 weeks simulated in situ use as determined by tensile tests using an Instron. All tests were performed in triplicate and each value represents the mean with standard deviation.

<table>
<thead>
<tr>
<th>Material</th>
<th>Load (N) (±StDev)</th>
<th>Tensile Stress (MPa) (±StDev)</th>
<th>Modulus (MPa) (±StDev)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control catheter before soaking</td>
<td>60.82 (± 6.68)</td>
<td>5.39 (± 1.29)</td>
<td>0.43 (± 0.045)</td>
</tr>
<tr>
<td>Antimicrobial catheter before drug elution</td>
<td>68.21 (± 3.66)</td>
<td>6.97 (± 0.39)</td>
<td>0.69 (± 0.16)</td>
</tr>
<tr>
<td>Control catheter after 12 weeks soaking</td>
<td>64.35 (± 3.42)</td>
<td>6.57 (± 0.35)</td>
<td>0.89 (± 0.15)</td>
</tr>
<tr>
<td>Antimicrobial catheter after 12 weeks drug elution</td>
<td>66.23 (± 4.01)</td>
<td>6.76 (± 0.41)</td>
<td>1.21 (± 0.48)</td>
</tr>
</tbody>
</table>

Despite a load of between 60 to 70 N being applied to the catheters they all failed to break, both before and after soaking in artificial urine. The tensile stress taken at the maximum point on the graph would be in excess of this if the Instron had larger parameters. Statistical analysis comparing control and antimicrobial material prior to 12 week aging in a simulated urine environment showed there to be no significant difference (p = >0.05) in load, tensile stress, elongation or modulus values. The parameters also remained the same (p = >0.05) between the two groups following 12 weeks soaking/drug elution. No significant difference (p = >0.05) was seen for all parameters when comparing antimicrobial catheters before and after drug elution or when comparing the load and tensile stress values between the control catheters before and after soaking. The only significant difference (p = 0.016) was in the modulus value obtained from control catheters before soaking compared to control catheters after soaking.
All catheters at the start of the tensile test were 273 mm in length. Following tensile tests, all elongated to the maximum limit on the instron of 1029.5 mm without breaking as depicted in Figure 6.4. This represents a 3.7 fold increase in length (377%). On release of the load, permanent deformation of both the control and antimicrobial catheters had occurred.

**Figure 6.4:** Typical extension (mm) of control and antimicrobial urinary catheters upon tensile testing, before and after 12 weeks soaking/drug elution in simulated urine.
6.4. DISCUSSION

The mechanical performance of urinary catheters is most important for patient safety. Boppart et al (2003) [216] reported on a Foley catheter which broke during clinical use. They report on a patient with a 100% silicone rubber Foley catheter in place who forcibly removed the catheter without deflating the balloon, fracturing it mid-way along its length and leaving the distal portion of the catheter remaining in the urethra. Although it is not common for urinary catheters to be placed under such extreme force, it is recognised that upon insertion, during use and removal, catheters and their balloons can be subject to significant alternating tensile forces. The importance of correct mechanical properties and the ability of modified catheters to withstand the daily forces it encounters to the level of existing catheters are paramount if patient safety is to be upheld.

Mechanical testing and modelling of a material for biomedical applications have to be based on conditions representative of the application of interest [217]. The behaviour of the inflated catheter balloon and catheter shaft when aged in simulated urine at 37°C can predict the functional performance during actual use in patients. In this study, using the ASTM, BS and FDA specifications as guidelines, the tensile testing of catheter shafts and properties of balloons in their prior - to - use state and representing in situ use through incubation in artificial urine was assessed. It is acknowledged, however, that this investigation would require further work and additional tests e.g. cyclic fatigue testing, to industrial standards in order truly evaluate the mechanical performance of the antimicrobial catheter. Testing of catheter balloons is important to ensure symmetrical inflation, no leakage and reliable deflation. Under inflation and asymmetrical inflation of balloons may cause
the catheter to slip out or result in leakage of urine around the catheter shaft. Upon removal of the catheter after the intended period of use, the balloon has to be fully deflated. On deflation, the balloon should ideally shrink back to its pre-inflated shape. This allows for easier removal of the catheter from the patient and minimal tissue damage [218]. Silicone catheter balloons are however prone to forming cuffs/ridges after their contents have been manually aspirated. Both control and antimicrobial balloons showed ridges upon deflation, though the catheter shaft diameter was less than 4 French units in size from the original so should not exhibit adverse effects to a patient upon its removal. All unions remained intact when challenged with 1 kg weight with no occlusion of the eyeholes or any rupture and leakage throughout the tests. Overall results obtained from tests performed on the antimicrobial balloons were the same as those from the control silicone balloons, which imply that they would be mechanically stable in clinical practice.

Tensile testing of the catheter shafts revealed no significant difference (p = >0.05) in load, tensile stress or modulus values when control catheters were compared to antimicrobial catheters before and after soaking/drug elution. The only significant difference (p = 0.016) observed was between the modulus value obtained when control catheters were compared to each other before and after soaking. An increase in the modulus can render the material less flexible and slightly more resistant to deformation [219]. Ramesh et al (2001) [220] evaluated the mechanical properties of three commercially available siliconized latex Foley catheters aged in artificial urine for 14 days. They report that tensile strength before aging was similar but following aging there was a considerable reduction in strength which they report may adversely affect the mechanical integrity of the catheter and breakage may happen
during the retrieval of the catheter from the patient. In this study, testing of the catheter following aging appeared to have no adverse effects on performance characteristics.

Schierholz et al (1997) [219], report that the incorporation of solvated rifampicin into swelling activated polydimethylsiloxane shunts at a concentration of 0.5% (w/w) resulted in a decreased tensile strength to 80% of the original and a decrease in elongation down to 62%. However they reported that this reduction had no restriction on their use in modern medicine as shunt systems. Similarly, van Noort et al (1979) [221] through manually incorporating gentamicin sulphate powder in the raw stock of room temperature - vulcanized silicone rubber found the tensile strength and extensibility to significantly decrease with increasing concentrations of gentamicin sulphate whilst the stiffness was comparatively little affected. Changes in the mechanical properties were not however considered to be great enough to affect the application of drug impregnated silicone rubbers to cerebrospinal fluid shunting systems. It is probable that a crystalline state of the drugs within the polymer and their angular shape may produce fracture points along the catheter and their release leave vacuoles and a honeycomb type matrix which could have caused the decrease in tensile properties of the material [221].

In this investigation, the impregnation of antimicrobial agents into the silicone catheter had no significant (p = >0.05) impact on the load, tensile strength and modulus values compared to the catheter without antimicrobial agents. Based on the parameters tested, this suggests that the mechanical performance of the antimicrobial catheter is equal to that of the catheter used as a control which is in clinical use. This
may be because the drugs are uniformly distributed within the polymer on a molecular scale and their release following 12 week incubation in solution, albeit leaving a slightly rougher surface, does not impact on the mechanical performance of the antimicrobial catheter.

Silicone is reported to have a low tensile strength, typically in the range of 1 - 8 MPa, a Young’s modulus typically 1 - 5 MPa and can elongate 200 - 1000% before breakage occurs [216, 220]. The low tensile strength and modulus of all sets of catheters mean that the material deforms easily. Both control and antimicrobial catheters reached the maximum length limit of the Instron without breaking and elongated to in excess of 377% of their original size. As the load was released a change in both the control and antimicrobial catheter shape and size causing them to become permanently deformed was observed. However, it is considered that urinary catheters being exposed to such forces whilst in situ is rare and as indwelling Foley catheters are not re-used the clinical relevance of the effect of the release following exposure to high loads is unclear. The ability to deform is what keeps the material flexible for manoeuvring through the urethra and from breaking and the antimicrobial catheter proved to be as effective as the conventional all silicone Foley catheter. The point at which permanent deformation occurred which may be of more relevance was not measured and is a point for future work.

The American, British and FDA standards specify the methodologies for testing the mechanical performance of catheters but literature on the varying types of Foley catheters used for LTC are limited and give differing experimental results. To determine an acceptable value for short - term and long - term urinary catheters
would be a helpful guide. Within the facilities available and the basic tensile and balloon tests performed, by correlating the performance of the all silicone Foley catheter used as a control with the antimicrobial catheter, experiments revealed no significant differences. This implies that the incorporation of antimicrobial agents into the catheter appears to equal the mechanical ability of the Foley catheter in clinical use and should therefore be suitable if applied to clinical practice.
CHAPTER 7

DISCUSSION AND CONCLUSIONS
7.0. DISCUSSION AND CONCLUSIONS

As modern day medicine becomes more reliant on the use of medical devices for diagnostic and therapeutic procedures, infection associated with them has become of major concern. Bacterial colonisation on biomaterial surfaces and the development of biofilms can make eradication of infection extremely difficult. Often to resolve the infection, removal of the device is necessary, as is the case with patients that are on long-term indwelling urinary catheterisation. It is almost inevitable that patients on LTC will at some point go on to develop a CAUTI and with the high prevalence rate, degree of morbidity and associated costs, new ways of reducing bacterial colonisation on catheter surfaces is warranted but producing urinary catheters that are refractory to microbial colonisation continues to be an uphill task. Little has changed in the design of the Foley catheter since it was first introduced and even with meticulous care, the system does not prevent bacteriuria and catheter colonisation in patients catheterised long-term. The best prevention is to minimise urethral catheter use and to seek device alternatives, such as intermittent catheterisation, suprapubic catheterisation, urinary diversion etc… whenever possible but this may not always be feasible. The need for an indwelling urinary catheter should be reassessed while managing patient care but for some patients discontinuing catheterisation is not an option and urinary catheterisation can continue for years or even for the duration of the patient’s life [222].

The molecular mechanisms of microbial adherence to surfaces and the development of new materials intrinsically resistant to colonisation along with host-pathogen interactions is an area that continues to be investigated [223] but until such time,
education into urinary catheter care, hand washing, correct aseptic techniques upon catheter insertion and the maintenance of a clean, closed drainage system continue to be important defences in protecting patients from bacterial invasion. In line with other research such as aganocide instillation into the bladder, retention balloons instilled with antimicrobial solutions, catheter valves able to be turned on and off to allow for a more natural flushing mechanism effect rather than a constant trickle, the development of novel antimicrobial biomaterials is an area which has gained interest in recent years.

There are now several options for antimicrobial catheter materials designed to reduce microbial colonisation. Evaluations of the antimicrobial urinary catheters in clinical practice have been mixed and duration of protective activity is short. At this time there are no antimicrobial urinary catheters available for long-term use, where the problem of infection is much greater. The intention of this study was, through the impregnation of antimicrobial agents into silicone urinary (Foley) catheters, to evaluate a suitable combination and concentration that would provide protective activity against colonisation by principal microorganisms involved in CAUTIs over a prolonged period (12 weeks) and which would help protect against the emergence of bacterial resistance.

After screening several antimicrobial agents, a combination of rifampicin, sparfloxacin and triclosan were impregnated into the catheter. Each having different modes of activity on bacterial cells was a way to extend the spectrum of activity and to investigate the ability of the drugs to provide protection against bacterial resistance. The SPTT used to monitor the potential duration of antimicrobial activity
against MRSA, CoNS, *E.faecalis, E.coli, K.pneumoniae* and *P.mirabilis* showed that activity continued for >100 days with the exception of *E.faecalis*. It was envisaged that if the organisms were susceptible to the individual antimicrobial agents, when the agents were combined they would act in synergy to provide a dual drug therapy which would help reduce the chances of resistance. The SPTT revealed that *E.coli, K.pneumoniae, CoNS* and MRSA had at least two drugs active on them over a prolonged course. Whether the size of the ZI relates to the antimicrobial material’s ability to provide protective activity against bacterial colonisation was not determined by the SPTT. Time - kill assays (tK100) were therefore developed to assess this. Exposing the material to urine to form a conditioning film is an important part of colonisation as bacteria may attach differently to conditioning films than bare material and XPS studies confirmed that urinary components (C, N, Na increased whilst the Si surface was masked) had adsorbed to the catheter surface. tK100 assays demonstrated that the antimicrobial catheter was able to kill 100% of all attached bacteria with the exception of ~25 - 50 cfu/mL *P.mirabilis* remaining within a 72 hour time frame. As the tK100 test is a static test that challenges the material with high numbers of organisms only once, an *in - vitro* model designed to test the catheters’ ability to prevent bacterial colonisation following repeated challenges under flow conditions was developed.

The *in - vitro* model proved the antimicrobial catheter to be capable of preventing colonisation by MRSA and *E.coli* for the targeted period of 12 challenges/weeks (84 days), *K.pneumoniae* and *P.mirabilis* for 8 challenges (50 days) and *E.faecalis* for 1 challenge (8 days). *E.faecalis* isolated from the effluent of the colonised catheters had rifampicin, triclosan and sparfloxacin MIC values which remained unchanged
from the original inoculum, suggesting that the susceptibility of *E. faecalis* to the agents as they released was not sufficient to prevent colonisation of the catheter. This was also implied by the short duration of activity indicated by the SPTT. The lack of activity against *E. faecalis* may have been due to the higher rifampicin, triclosan and sparfloxacin MICs in the first instance and it was also noted that *E. faecalis* grew more favourably in TSB than in artificial urine (Appendix 2) which may have favoured the growth of *E. faecalis* in the *in vitro* model tests. The *E. faecalis* strain used in this study had an antimicrobial susceptibility profile that was typical of the clinical isolates collected and with a high proportion of *E. faecalis* being isolated from the urine of patients on LTC, the catheters lack of activity against it could be of concern, although its clinical implication with regards symptomatic CAUTI is a point for further investigation.

The SPTT showed a >100 day duration of activity towards *P. mirabilis* with activity mostly being supplied by triclosan, but as the antimicrobial material did not clear 100% of attached *P. mirabilis* in tK100 tests it was unclear how protective the catheter would prove upon repeated challenges with this organism in an *in vitro* model. Catheters became colonised with *P. mirabilis* on the 8th (day 50), 11th (day 71) and 12th (day 83) challenge respectively with the MIC of triclosan increasing marginally from 0.094 \( \mu g/mL \) to a maximum of 0.38 \( \mu g/mL \). This is however still in the sensitive range. The SPTT showed triclosan and sparfloxacin to be active against *K. pneumoniae*. Catheters became colonised with *K. pneumoniae* following the 8th (day 50) and 9th (day 57) challenge with the MIC of sparfloxacin and triclosan increasing up to 2 \( \mu g/mL \), which is approaching more resistant concentrations. The average concentration of sparfloxacin released from the catheter was approximately
0.07 mg/catheter/day (or 1.25 µg/mL/day) following the initial burst effect and approximately 0.15 mg/catheter/day (or 2.4 µg/mL/day) of triclosan during the 28 day drug release study and these concentrations are above the concentration required to inhibit *K. pneumoniae* and *P. mirabilis*. In addition, ToF-SIMS also revealed that the distribution of sparfloxacin and triclosan on the catheter surface was mostly evenly distributed, which is important for uniform drug release but some drug localization was observed. CAUTIs can be caused by bacteria gaining access to the catheter and bladder at the time of catheter insertion. It might be assumed that the drug localization could add to the initial higher delivery (burst effect) of antimicrobial agents which could help reduce bacterial adherence and the early onset of infection [194]. If the drug release trend was to remain stable as the SPTT suggests, with 80.2% sparfloxacin and 70.1% triclosan remaining there should have been sufficient drugs to protect from colonisation over the 12 weeks. It was expected that at the concentrations being released this would place the drugs above the MIC but below the MPC and hence within the MSW, but if two drugs released at concentrations above the MIC and superimposed each other resistance would not be seen as bacteria would require two mutations for them to grow in the presence of the antimicrobial agents. To fully establish if the dual drug principle worked, the *in vitro* model would need to be performed with catheters impregnated with drugs individually and bacterial colonisation and resistance compared to catheters impregnated with all three agents to observe if resistance to individual drugs is generated more readily than combinations of drugs.
Why the catheters became colonised with *K. pneumoniae* and *P. mirabilis* but not MRSA and *E. coli* is unclear but several reasons are proposed. The device is not capable of inhibition of microbial adherence per se, but the killing of already adherent microorganisms. SEM images of the *P. mirabilis* and *K. pneumoniae* colonised antimicrobial catheters revealed extensive biofilm formation and it is possible that by chance a few bacteria remained attached to the catheter during the latter *in-vitro* challenges in spite of the concentrations of drugs being released from the catheter surface. These may have then rapidly multiplied and started to become phenotypically insusceptible to the drugs. If bacteria were sonicated from the catheter lumens and cultured, it is expected that the MICs would have been higher than from the planktonic samples taken from the effluent. SEM images of the antimicrobial catheters challenged with MRSA and *E. coli* showed the presence of what appeared to be remnants of dead bacteria and although these passed the 12 week *in-vitro* challenge, it could indicate that dead cells may act as anchor points and promote subsequent bacterial colonisation as was exhibited by *P. mirabilis* and *K. pneumoniae*. A further reason may be that as the flow rate was decreased when the *in-vitro* model was left for a period of time the decrease in flow rate and shear may have encouraged any bacteria if attached to the catheter surface to multiply. Their presence on the catheter surface and in the perfusion fluid despite the antimicrobial agents may have overwhelmed the catheter, causing bacteria to permanently attach to the surface and survive to change phenotype. The drugs would therefore not be inhibitory to biofilms. AFM also revealed that the roughness of the catheter surface appeared to be significantly greater as the drugs eluted which may have caused some bacteria to adhere to parts of the abraded surface which may have
initiated the initial stage of bacterial colonisation. Further testing would however be required to prove or disprove this theory.

Blanco et al (2009) [224] reported that the elution of triclosan from polyglycolide sutures was enhanced when a serum medium was used because the presence of proteins and lipids increased the solubility and elution of triclosan. A further reason for bacterial colonisation may be that the elution of triclosan, sparfloxacin and rifampicin under flow conditions in TSB could have produced a greater degree of drug release than that shown through the diffusion into water upon which drug release measurements were taken. Due to the flow rates that the catheters were under it is possible that this could cause a greater degree of drug diffusion into the surrounding medium, making concentrations released between weeks 8 - 12 below the MIC or minimal bactericidal concentration (MBC) of the organisms. If drug release was greater in the in - vitro setting the concentrations may not have been sufficient to prevent colonisation. Drug studies also revealed that triclosan and sparfloxacin release at different rates, and if in the latter stages during the in - vitro challenge, one drug fell below the MBC necessary to kill K.pneumoniae or P.mirabilis, resistance to one drug may have occurred followed by resistance to the second agent which could have resulted in colonisation of the catheter. This may be logical for the case of K.pneumoniae where both sparfloxacin and triclosan MIC values increased. That being said the antimicrobial catheter prevented the colonisation of E.coli which had very similar MIC values, SPTT and tK100 results to K.pneumoniae.
The situation where the antimicrobial catheter could have the most beneficial effect is in patients who are symptomatic, where relapses are common and more frequent catheter removal and replacement is normally required. By removing the catheter the biofilm is removed and patients will usually be prescribed a course of antibiotics to kill residual bacteria in the bladder. It is hoped that the antimicrobial catheter would protect against colonisation from principal invading organisms to an extent that a patient would not experience symptoms associated with infection. In patients with repeat symptomatic CAUTIs opposed to newly catheterised patients it may be necessary to treat residual organisms in the bladder with an appropriate course of antibiotics prior to insertion of the antimicrobial catheter to reduce bacterial numbers. This may be helped by the catheter having been proved to have an antimicrobial burst effect which should prevent the early onset of infection at the time of catheter insertion. With 10% of catheterised patients showing symptoms associated with infection, if the antimicrobial catheter with further research proved to be as effective against other problematic bacterial species and strains then a considerable saving in hospital time and treatment and vastly improved quality of life for patients could be achieved.

Whether the use of the catheter will replace susceptible bacteria with those that are resistant to the agents in the catheter and just as many infections occur but with resistant organisms is a question that needs to be asked. This cannot be fully answered until clinical trials take place but the use of other antimicrobial catheters has provided an insight. A study by Wong et al (2010) [225], compared the effectiveness of the antimicrobial Bactiseal EVD (impregnated with 0.15% w/w clindamycin and 0.056% w/w rifampicin) with plain conventional EVDs coupled
with systemic antibiotics in terms of nosocomial infection and cerebrospinal fluid (CSF) infection. They found the proportion of patients with nosocomial infections and secondary outcomes such as CSF infection in the antimicrobial catheter group was not statistically different from the group of patients receiving the conventional catheter with antibiotics. There was also no significant difference between the two groups in the incidence of resistant infections or in the named resistant infections. Interestingly even though the Bactiseal agents are not active upon Gram negative bacteria, there was no evidence of an increase in the absolute number of these infections. The antibiotic impregnated EVD proved to be as effective in reducing infections as the conventional EVD with systemic antibiotics and this could also be the case for the antimicrobial urinary catheter. In respect of the clinical impact of the long-term antimicrobial urinary catheter it should also reduce the need for the consistent use of oral antibiotics prescribed to some symptomatic patients. Frequent use of systemic antibiotics can lead to an environment of more resistant microbial populations and their overuse is always a concern with regards to resistant opportunistic infections e.g. *Clostridium difficile*. The study by Wong *et al* (2010) [225] supports that the dangers of systemic antibiotics could potentially be avoided by the use of an antimicrobial catheter. The less frequent use of systemic antibiotics with the use of an antimicrobial urinary catheter should make the use of systemic antibiotics more effective when they are really needed.

In addition to the microbiological testing of the antimicrobial urinary catheter, the low concentrations of antimicrobial agents in the catheter caused no adverse effects to the mechanical performance of either the catheter shaft or balloon. In clinical
practice the catheter would therefore be expected to be as mechanically stable as the control catheter used in this study.

This preliminary study suggests that, with further work, the catheter has the potential to have a positive impact on reducing bacterial colonisation. With little prior information regarding the concentration of antimicrobial agents in the chloroform that become incorporated into the silicone, the impact that drug loading has upon drug release is an area that requires further investigation. In particular the concentration of rifampicin could be increased so that it has a more prolonged and powerful effect against Gram positive bacteria (e.g. staphylococci) and increased to a level that is in line with the release of sparfloxacin and triclosan. Kohnen et al (2003) [118], reported on drug optimisation that achieved a 1:1 ratio of rifampicin and sparfloxacin release which remained stable for at least 40 days. Optimisation of drug release so that concentrations are higher and the release ratio of each is the same may deliver the agents in a more sustained manner which could reduce bacterial colonisation over the targeted 12 weeks and beyond and help reduce bacterial resistance. To repeat the in - vitro model with more replicates and to test the catheters against a larger array of microorganisms is necessary to obtain a broader view of its ability to prevent colonisation. To make the in - vitro model more clinically predictive, a method would need to be devised to test the ability of the catheters to prevent bacterial colonisation using real human urine. Any alteration to drug content and release would require adjacent in - vitro model testing and to gather more precise drug release information it may be necessary to collect effluent from the antimicrobial catheters, as they are under flow conditions in the in - vitro model, and measure drug release concentrations over a period of 12 weeks. The effect of
storage on the stability of the antimicrobial agents and shelf life of the device must be determined as the loss of activity could cause failure when used. Sensitivity, allergic reaction and toxicity testing may also be required to assess local and systemic effects of exposure of the antimicrobial agents to the urinary tract environment prior to clinical trials. Attention must also be paid to the ability of the antimicrobial catheter to prevent encrustation by urease - producing organisms like \textit{P.mirabilis}. A bladder model similar to the design by Stickler \textit{et al} (1999) \cite{7}, could be used to assess this and atomic absorption spectroscopy (AAS) employed to examine mineral deposition.

The eventual aim is to progress the catheter to a stage in which it can be trialled \textit{in vivo} and in the form of a small - scale clinical trial. With the optimisation of drug release to overcome the potential for bacterial resistance, the testing of more organisms in the \textit{in vitro} model including trials with human urine and encrustation studies, it is considered that the catheter will show promise in providing protection against bacterial colonisation over a long duration. If the new catheter proves in clinical trials to reduce bacterial colonisation, encrustation or to extend catheter life, then a significant impact could be made on reducing CAUTIs in patients on LTC.
REFERENCES


Appendices

APPENDICIES

Appendix 1: Agar incorporation protocol for determining MIC of bacterial strains to sparfloxacin and triclosan

1. 0.03 g of sparfloxacin and/or triclosan were weighed out and placed in 50 mL of ethanol to dissolve and 50 mL of sterile distilled water to make a concentration of 300 µg/mL.
2. A 1:10 dilution was performed to make a concentration of 30 µg/mL.
3. Serial dilutions were made to cover a concentration ranging from 0.08 - 30 µg/mL (as shown below).
4. 2 mL of each concentration was added to 18 mL of molten ISA to give a further 1:10 dilution to yield a final concentration ranging from 0.008 - 3 µg/mL and poured into petri dishes.
5. Plates were swirled well to evenly distribute the antimicrobial agents and left to set.

<table>
<thead>
<tr>
<th>Initial Concentration (µg/mL)</th>
<th>Amount taken from previous concentration (mL)</th>
<th>Amount of water added (mL)</th>
<th>Final Concentration (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>20</td>
<td>13.33</td>
<td>6.67</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>10.0</td>
<td>10.0</td>
<td>1</td>
</tr>
<tr>
<td>7.5</td>
<td>15.3</td>
<td>5.0</td>
<td>0.75</td>
</tr>
<tr>
<td>5</td>
<td>13.33</td>
<td>6.67</td>
<td>0.5</td>
</tr>
<tr>
<td>3.8</td>
<td>15.20</td>
<td>4.8</td>
<td>0.38</td>
</tr>
<tr>
<td>2.5</td>
<td>13.15</td>
<td>6.85</td>
<td>0.25</td>
</tr>
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<td>1.9</td>
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</tr>
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<td>6.85</td>
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</tr>
<tr>
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</tr>
<tr>
<td>0.64</td>
<td>13.60</td>
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<tr>
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<td>15.00</td>
<td>5.0</td>
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</tr>
<tr>
<td>0.08</td>
<td>13.33</td>
<td>6.67</td>
<td>0.008</td>
</tr>
</tbody>
</table>
Appendix 2: Growth Curves

Growth curve of MRSA in TSB and artificial urine

Growth curve of *E. faecalis* in TSB and artificial urine
Growth curve of *P. mirabilis* in TSB and artificial urine

Growth curve of *K. pneumoniae* in TSB and artificial urine
Appendix 3: Drug Release

Concentrations (µg/mL) of sparfloxacin and triclosan released from the antimicrobial urinary catheter over a 28 day elution period. Each value represents the average of three replicates with standard deviations.
Appendix 4: XPS scans from conditioned and non-conditioned control and antimicrobial catheter material

XPS wide scan from non-conditioned control material

XPS wide scan from conditioned control material

XPS wide scan from non-conditioned antimicrobial material

XPS wide scan from conditioned antimicrobial material
XPS high resolution scan of O 1s peak from non-conditioned control material

XPS high resolution scan of O 1s peak from conditioned control material

XPS high resolution scan of O 1s peak from non-conditioned antimicrobial material

XPS high resolution scan of O 1s peak from conditioned antimicrobial material
Appendices

XPS high resolution scan of Ca 2p peak from non-conditioned control material

XPS high resolution scan of Ca 2p peak from conditioned control material

XPS high resolution scan of Ca 2p peak from non-conditioned antimicrobial material

XPS high resolution scan of Ca 2p peak from conditioned antimicrobial material
XPS high resolution scan of Cl 2p peak from non-conditioned control material

XPS high resolution scan of Cl 2p peak from conditioned control material

XPS high resolution scan of Cl 2p peak from non-conditioned antimicrobial material

XPS high resolution scan of Cl 2p peak from conditioned antimicrobial material
Appendicies

XPS high resolution scan of Mg 2p peak from non-conditioned control material

XPS high resolution scan of Mg 2p peak from conditioned control material

XPS high resolution scan of Mg 2p peak from non-conditioned antimicrobial material

XPS high resolution scan of Mg 2p peak from conditioned antimicrobial material
Appendices

Appendix 5: The composition of simulated urine used in the urinary catheter mechanical property testing

Artificial Urine from BS EN 1616 - 1997 [173, 214]

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Mass (g)</th>
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</thead>
<tbody>
<tr>
<td>Urea</td>
<td>25</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>9</td>
</tr>
<tr>
<td>Disodium hydrogen orthophosphate, anhydrous</td>
<td>2.5</td>
</tr>
<tr>
<td>Ammonium chloride</td>
<td>3</td>
</tr>
<tr>
<td>Potassium dihydrogen orthophosphate</td>
<td>2.5</td>
</tr>
<tr>
<td>Creatinine</td>
<td>2</td>
</tr>
<tr>
<td>Sodium sulfite, hydrated</td>
<td>3</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000</td>
</tr>
</tbody>
</table>

Chemicals (Sigma-Aldrich) were accurately weighed out and dissolved in 1 L of distilled water. The artificial urine was sterilised by filtration through a 0.2 μm pore and pH was recorded at approximately 6.6.