Emerging Medical and Engineering Strategies for the Prevention of Long-Term Indwelling Catheter Blockage

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Abstract

Urinary catheters have been used on an intermittent or indwelling basis for centuries, in order to relieve urinary retention and incontinence. Nevertheless, the use of urinary catheters in the clinical setting is fraught with complication, the most common of which is the development of nosocomial urinary tract infections (UTIs), known as catheter-associated urinary tract infections (CAUTIs). Infections of this nature are not only significant owing to their high incidence rate and subsequent economic burden, but also to the severe medical consecutions that result. A range of techniques have been employed in recent years, utilising various technologies in attempts to counteract the perilous medical cascade following catheter blockage. This review will focus on the current advancement (within the last 10 years) in prevention of encrustation and blockage of long-term indwelling catheters both from engineering and medical perspectives, with particular emphasis on the importance of stimuli-responsive systems.

Keywords

Catheter-associated urinary tract infection, *Proteus mirabilis*, biofilm, hydrogel, bacteriophage

Introduction

The Clinical Problem: CAUTI

Of the approximately 100 million catheters sold annually worldwide,[1] the most commonly employed in the healthcare setting is the Foley indwelling urinary catheter, which constitutes a flexible silicone or latex tube (inserted into the bladder via the urethra), held in place by an inflatable balloon and connected to a drainage bag, thus completing the sterile closed-drainage system. In the UK alone, complications arising from the use of Foley catheters cost the National Health Service (NHS) between £1.0-2.5 billion, and accounts for approximately 2100 deaths annually.[2] CAUTIs constitute 80% of all nosocomial UTIs, thus comprising over 40% of all nosocomial infections in the USA alone.[3,4]

Despite innate mechanical safeguards against microbial invasion of the urinary tract, specific pathogens are capable of colonising and thriving in this environmental niche. Within the healthy, uncatheterised urinary tract, the regular flushing of the urethra as a result of bladder emptying helps to impede ascending infection of the urinary tract by mechanical means.[5] Further biological safeguards such as the lining of the bladder with urothelial cells (covered with a glycosaminoglycan mucin), also helps to mitigate infection by resisting adherence via the activation of microbial-sensing proteins, thus triggering the host defences with a cascade of cellular and molecular effectors.[6] Catheterisation of the urinary tract provides opportunities that may be exploited by uropathogenic species for avoidance of such safeguards, thus resulting in successful colonisation and an increase in patient susceptibility to UTIs.[7] The majority of opportunistic uropathogens are faecal contaminants or skin residents from the patient's own native or transitory microflora, originating in the periurethral area.[8,9] Bacterial entry into the bladder may occur at the point of catheterisation, through the catheter lumen, or along the catheter-urethral interface.[10] Approximately two thirds of bladder colonisation during CAUTI occurs via the extraluminal route, where organisms ascend from the urethral meatus, via the catheter/urethra interface. Less common is bladder colonisation via the intraluminal route (approximately one third of CAUTI), where bacterial cells transfer into the bladder as a result of manipulation of the sterile closed drainage system.[11,12]

Catheter-associated bacteriuria (CAB) is defined as the presence of $\geq 10^5$ colony forming units per millilitre (CFU/ml) of one or more bacterial species in a single catheter urine sample. It is generally universal in patients undergoing long-term indwelling catheterization, and is detected in the majority of patients who have been catheterised for > 1week.[13] Fortunately, most cases of CAB are asymptomatic and are thus commonly referred to as catheter-associated asymptomatic bacteriuria (CAASB). Antibiotic prophylaxis is not recommended for CAASB, although it may postpone biofilm infections for 1-2 weeks.[14] Indeed, the ECDPC recently reported that the most common isolates from CAUTI are resistant to at least one of the antimicrobial agents commonly used in clinical practice. Namely, 26.3% of *E. coli* isolates are resistant to third-generation cephalosporins, 26.6% of *P. aeruginosa* isolates are resistant to ceftazidime ,and 9.5% of *Enterococcus* sp. Isolated are resistant to vancomycin.[15]

In contrast, the term CAUTI is used to refer to individuals suffering from symptomatic infection that is significant bacteriuria as well of symptoms of infection in the absence of any other identifiable sources. Although fewer than 3% of patients with CAASB develop bacteraemia, given the prevalence of infections of this nature, CAUTI is one of the most common causes of secondary bloodstream infection in acute care facilities. Additionally, CAUTI is the source of over 50% of episodes of blood stream infections within the long-term care setting.[16]

History and Development of the Foley Catheter

Derived from the ancient Greek *kathiénai*, the word *catheter* can be literally translated as "to thrust into", or "to send down", and is used to describe an instrument used to drain fluid from a body cavity. Since its initial inception into human medicine more than 3500 years ago, a vast array of materials and designs have been employed to drain the dysfunctional bladder. Early documentation reveals that metals (such as copper, tin, bronze and gold) were used by the Greek physiologist Erasistratus in the third century B.C.[17] Egyptians utilised lead and papyrus in the design and manufacture of early catheter models,[18] whilst the Chinese in 100 B.C. exploited organic materials such as palm leaves, dried reeds or hollow onion stems that were lacquered as urological devices.[19] The first example of a flexible catheter made of malleable gum-elastic was created by Bernard (a French jeweller and goldsmith) in the late

18th century, following the development of natural rubber in 1735.[17] Difficulties in achieving a compromise between rigidity and flexibility were not overcome until the vulcanisation of rubber was discovered by Charles Goodyear in 1839. [19]The concept of a catheter retention balloon was introduced in 1853, using rubber or woven fabric dipped in linseed oil and baked.[20] The modern-day Foley catheter, used within the acute care and community settings today, was first introduced in the mid-1930s by Dr. Frederick B. Foley.[21] Despite being one of the most commonly used medical devices employed within modern medicine, and the cornerstone for the management of urinary incontinence, the Foley catheter has opened a Pandora's Box of medical complications, owing to its inherent design flaws and vulnerability to rapid-onset bacteriuria.[5]

Long Term Indwelling Catheters

Duration of catheterisation is the most important risk-factor associated with CAUTI. Approximately 10-50% of patients undergoing short-term or intermittent catheterisation (1-7 days) develop bacteriuria, whereas catheterisation for >28 days often results in the development of CAUTI.[22] Encrustation and blockage following infection by *Proteus mirabilis* (*P. mirabilis)* is a common complication associated with urinary devices, and is a major cause of morbidity and mortality in CAUTI.[23]

Proteus mirabilis: Pathogenesis and Mechanisms of Catheter Encrustation

P. mirabilis are Gram-negative members of the family *Enterobacteriaceae*, distinguishable from almost any other genera by their striking ability to swarm across a solid surface.[24] Indwelling urinary catheters serve as the ideal initiation site of infection by *P. mirabilis*. This pathogen has been shown to have the greatest ability to attach to catheter surfaces (including ethylene, propylene, polystyrene, sulfonated polystyrene, silicone and red rubber catheters) than any other Gram-negative organism.[24,25] There is strong epidemiological and experimental evidence that *P. mirabilis* is the major cause of catheter encrustation,[26] and has been found to colonise approximately 40% of all chronic indwelling catheters.[16]

Within the catheterised urinary tract, microorganisms not only live as planktonic cultures of dispersed single cells, but tend to accumulate at interfaces to form polymicrobial aggregates known as biofilms. Biofilms comprise communities of surface-attached cells, embedded into an extracellular polymeric matrix. Biofilm formation occurs as a result of complex intra- and intercellular signalling and coordinated communication processes, mainly regulated by the quorum sensing system ubiquitous in the bacterial world.[3,27] Biofilms initially formed on the catheter surface tend to be monomicrobial, although often develop into polymicrobial structures during long-term catheterisation, with up to 72% of catheters being colonised by two or more species,[28] including combinations of *P. mirabilis*, *Morganella morganii*, *Providencia stuartii*, *Escherichia coli* (*E. coli)*, *Pseudamonas aeruginosa (P. aeruginosa)* and *Klebsiella pneumoniae*.[29–31]

Although *P. mirabilis* is generally not the first organism found to colonise the catheter surface, it is common and of great significance in long-term catheterisation (Figure 1). Indeed, the most frequent causative agent for UTI in humans (approximately 80%), and one of the most common causes of Gram-negative bacteraemia in hospitalised patients is *E. coli*. [3] The longer a catheter is in place, the more like it is to become colonized by *P. mirabilis*. Since *P. mirabilis* biofilms are both more copious and more likely to cause blockage than those of other species, the focus of this review will henceforth be on infection, biofilm formation, and subsequent blockage of catheters following infection by this specied in

particular.[16] *P. mirabilis* is able to express a wide variety of virulence factors, perhaps the most relevant of which in terms of CAUTI initiation and establishment is the expression of a potent urease enzyme; a cytoplasmic nickel metalloenzyme whose upregulation is initiated by a shift to host body temperature and high concentrations of urea.[32,33]Urease induces the hydrolysis of urea to ammonia and carbon dioxide, thus elevating urinary pH and facilitating the precipitation of polyvalent ions within the urine. The resultant formation of struvite (magnesium ammonium phosphate) and apatite (calcium phosphate) crystals typically occurs when the urine pH is elevated to ~8.2,[34] and such crystals may then accumulate within the biofilms on the external and luminal catheter surfaces, obstructing urine flow and leading to complications such as incontinence and painful distention of the bladder (owing to urinary retention). This in turn leads to severe sequelae such as vesicoureteral reflux, bacteriuria, and ascending infection resulting in possible pyelonephritis and septicaemia.[30]
Furthermore, *P. mirabilis* is capable of producing an arsenal of bacterial toxins, two of which (haemolysin (HpmA) and *Proteus* toxic agglutinin (Pta), are involved in the process of tissue damage and renal migration, thus inducing acute pyelonephritis.[35,36] HpmA is a Ca^{2+}-dependent pore-forming cytolysin, which achieves destabilisation of the host cell by inserting into the cell membrane, causing Na^{+} efflux. Pta is a surface-associated cytotoxic protease, functional only at the high urinary pH consequential of *P. mirabilis* urease. It achieves bladder and kidney damage via damage to the structural integrity of the native cells. In the proposed mechanism of action, Pta punctures the host cell membrane, inducing cytosol leakage, osmotic stress and depolymerisation of actin filaments.[4,37]

[Insert Figure 1]

This review endeavors to present a range of novel approaches to biofilm reduction and eradication. The emphasis is mainly on techniques that are not yet clinically tested, but nevertheless have shown significant promise in lab-based trials for the application of delay/prevention of catheter blockage following infection by *Proteus mirabilis*. Some methods discussed may have not been utilized in the field of catheter encrustation to date, although demonstrate many aspects of transferable technology which may prove useful for the future of CAUTI management.

Part I: Engineering Approaches

Passive Release Coatings

Release-based coatings exert their antibacterial activity by leaching antibacterial compounds over time, exposing and subsequently killing bacterial cells both adhered and planktonic. In contrast to traditional methods of administering antibacterial agents, release coatings provide the ability to deliver agents at a high local concentration, whilst limiting systemic exposure and toxicity. Passive release of antimicrobial agents aims to achieve effective prophylaxis as well as potential treatment of established bacteriuria, although the reservoirs of antimicrobial cargos are inherently limited, thus their action is ultimately temporary. Unlike the stimuli-responsive approaches discussed in Part III, passive release kinetic profiles generally follow first- or second- order kinetics, where an initial burst release is followed by a decreasing tail distribution.[38] The timeframe of the concentration decline is highly application-dependent, although generally varies from hours to days, providing a significant challenge in coating design, specifically compromising between medical device function and sufficient cargo concentration within the carrier matrix. To date, the design of coatings that maintain

antimicrobial concentrations throughout the therapeutic window, sufficient to kill bacteria but low enough to limit eukaryotic cytotoxicity, remains a significant challenge.[39]

Emerging Developments in Catheter Coatings

Over the past decade, imbibing or coating medical implants in antimicrobial solutions has been commonly used to control infection. A broad range of passive release-based systems have been explored for the prevention or delay of urinary catheter encrustation and blockage, the most common of which is simple impregnation, where the agent is held within a reservoir and released via diffusion. The lack of a specific bonding mechanism results in rapid liberation from the release matrix, which generally utilises a polymeric carrier, for example poly(methacrylic acid) (PMMA), poly(vinyl alcohol) (PVA), poly(acrylic acid) (PAA), or poly(lactic-*co*-glycolic acid) (PLGA).[40]

Other approaches to achieve antimicrobial coatings on urinary catheters whilst avoiding the use of a polymeric reservoir include deposition by sonochemcial methods. Shalom *et al*[41] described the coating of urinary catheters with Zn-doped CuO nanoparticles using a high energy ultrasonic method, which displayed promising antibiofilm activity and biocompatibility as indicated by low *in vitro* cytotoxicity and negligible associated cytokine secretion. Evaluation of coated catheters *in vivo* using a rabbit model showed that rabbits catheterised with uncoated catheters scored positive for CAUTI after 4 days. In contrast, rabbits with coated catheters did not exhibit CAUTI until day 7 or remained uninfected for the entire duration of the 7-day experiment.

Nitric oxide (NO), a potent biofilm inhibitor, has also been exploited for use in indwelling medical devices.[42–44] Colleta *et al*[45] has investigated the impregnation of commercially available silicone Foley catheters with S-nitroso-N-acetyl-D-penicillamine (SNAP) via solvent swelling. SNAP acts a synthetic NO donor, exhibiting long-term NO release and stability when incorporated into low-water uptake polymers. Cytotoxicity testing using a mouse fibroblast cell-line showed that SNAP-impregnated catheters were fully biocompatible, whilst assessment of prototype catheters against 3-day *P. mirabilis* biofilms showed a 3-logarithmic reduction in cell viability when compared with control catheter segments. SNAP- doped catheter tubing was able to substantially decrease bacterial viability at each stage of biofilm maturation, thus making it, at the time of writing, the first example of the use of a synthetic NO donor capable of preventing mature *P. mirabilis* biofilm formation, whilst producing stable NO at nontoxic fluxes.

Hydrogels

Hydrogel coatings have also been employed as drug delivery systems for the potential release of therapeutic agents from the catheter surface. Hydrogels are ubiquitous throughout the literature and widely accepted as being suitable carrier matrices for the delivery of antimicrobial compounds. Hydrogels are cross-linked, insoluble, hydrophilic polymer networks capable of entrapping significant volumes of water as their liquid component. Hydrophilic coating of catheters provides a number of advantages over standard silicone catheters, including reduced patient discomfort, increased lubricity and reduced encrustation as a result of decreased microbial cell adhesion at the tissue-biomaterial interface.[46,13,47] Hydrogels have been shown to increase aggregation of planktonic cells and newly-nucleated crystals, leading to accelerated catheter blockage, although the addition of active agents is able to suppress this negative effect.[48]

Recently, preparation of hyaluronic acid (HA)-based hydrogels crosslinked with poly(methyl vinyl ether-alt-maleic acid) by thermal and microwave processes were evaluated as drug delivery systems using methylene blue (MB) as a model drug. Synthesised hydrogels showed a high affinity for MB, and were capable of sustained release over a period of 48 hours.

Additionally, *in vitro* microbiological assessment of the HA-based hydrogels demonstrated resistance to microbial adherence (greater than one-logarithmic reduction when compared with a PVC control) when evaluated with representative nosocomial strains of *Staphylococcus aureus (S. aureus)* and *P. mirabilis*.[49]

Anti-Adhesion / Antifouling Approaches

Approaches designed to prevent adhesion of bacteria generally aim to impede biofilm formation in its infancy using non-cytotoxic mechanisms (either by unfavourable surface topography or surface chemistry). Bacterial adhesion to biomaterial surfaces such as urinary catheters is generally described using a two stage model: initial, rapid, reversible adhesion (stage 1), mediated by non-specific physicochemical interactions, followed by secondary 'locking' adhesion (stage 2) involving specific bacterial-adhesion proteins.[50] Since the host-derived conditioning film of urinary components begins development upon insertion of the Foley catheter, any existing surface properties of the material are often concealed, and pathogen attachment promoted. Therefore, development of antifouling coatings must target not only uropathogen attachment but urinary constituents as well. Whilst the conceptual simplicity and potential biocompatibility of anti-adhesion coatings makes them an appealing approach, their antibacterial action requires extremely close proximity with bacterial cells. Surfaces such as catheters may become rapidly contaminated with non-specifically attached materials or dead cells, thus compromising the defect-free surface. Consequently, large-scale production and subsequent handling may prove challenging in future.

Poly(ethylene glycol) (PEG)-Based Approaches

The field of antifouling polymers is fast-growing, with new polymer structures being frequently designed for this specific purpose.[51] Poly(ethylene glycol) (PEG) has been the most widely used polymer for antifouling in recent years,[52,53] although its efficacy is dependent upon the surface grafting technique and polymeric architecture.[54] Development of anti-fouling coatings of marine mussel adhesive protein mimics conjugated to 3,4-dihydroxyphenyl-$_L$-alanine-modified poly (ethylene glycol) (mPEG-DOPA(3)) was shown to resist both urinary film formation and uropathogenic attachment *in vitro* when tested with a number of relevant isolates on TiO_2 disks,[55] although further randomised and large-scale clinical studies are necessary to determine the validity of these coatings.

Zwitterionic Approaches

Zwitterionic materials, containing equal positive and negative charge functionalities, maintain electric neutrality and are well-known for their superior antifouling properties.[56,57] Zwitterionic coatings have recently been applied to polydimethylsiloxane (PDMS), the material most commonly used for the production of silicone Foley catheters.[58–60] The incorporation of silver into zwitterionic coatings provides an additional aspect to antifouling coatings of this nature, and has been under recent investigation in several, broad scientific disciplines including membrane processes for water treatment and desalination.[61] The loading of zwitterionic polymer brushes with Ag^+ precursor ions, followed by their *in situ* reduction to Ag nanoparticles by UV irradiation was investigated by Hu *et al*.[62]The obtained organic-inorganic hybrid was capable of killing *E.coli* (>99.8% in 1 hour) upon contact with embedded silver nanoparticles, and subsequently releasing dead bacterial cells under wet conditions, preventing the associated immune response and blocking of the antimicrobial function. Similar technology has also been assessed in the terms of wound healing,[63] via impregnation of synthesised *in situ* silver nanoparticles into an antifouling zwitterionic hydrogel, thus eliminating the need for additional chemical reductants or toxic solvents. Such

applications may also be transferrable to the field of urinary catheters, via coating of the outer or inner luminal surfaces with such antimicrobial and antifouling hydrogels.

Surface Topographical Approaches and Nanostructured Materials

The material and resulting surface topography from which a urinary catheter is made can significantly impact the rate of biofilm formation. Latex catheters have a far more uneven surface topography than their silicone counterparts, which promotes microbial adherence, thus accelerating biofilm formation.[64] Recent studies have aimed to investigate and exploit the abilities of a material's surface topography in order to modulate cellular adhesion. Patterned surfaces, such as Sharklet™ have shown great promise of being a benign surface treatment for the prevention of bacterial attachment.[65,66] Reddy *et al*[67] assessed the ability of uropathogenic *E. coli* to colonise three variations of the Sharklet micropattern compared to a standard silicone control. All three variations outperformed controls in the prevention of *E. coli* colonisation. An average of 47% reduction in colony-forming units and bacterial area coverage was observed, in addition to 77% reduction in colony size in both tryptic soy broth (TSB) and artificial urine.

Contact Killing and Non-Release-Based Approaches

Contact killing approaches have gained attention in recent years owing to their ability to circumvent the issue of reservoir exhaustion synonymous with antibacterial agent release coatings. Instead, antimicrobial compounds are covalently anchored to the material surface via polymeric linkers. Bacterial attachment and proliferation is subsequently hindered by such compounds, generally as a result of disruption to the cell membrane via physical lysis or charge disruption.[39]

Catheter Surface Functionalisation: Plasma Deposition

Among methods of antibacterial surface modification of abiotic surfaces, plasma modification plays a rare but increasingly relevant role. With requirements for methods of covalent attachment alternative to solvent-based methods, plasma presents an attractive alternative deposition method which combines ease of preparation and versatility with economical and solvent-free processing. Plasmas, often referred to as the fourth state of matter, are typically generated via the ionisation of a gas by electrical discharge, forming charged particles including ions, electrons and radicals. The species generated exhibit a strong collective response to an applied magnetic field, thus interacting with and modifying surfaces via etching, implantation, deposition or functionalisation.[39]

Applications of plasma-treated surfaces per se, to deter bacterial attachment have generally not proved promising, as the arsenal of chemical species available via plasma deposition is limited and does not compete with the complexity of the bacterial attachment process.[68] A number of studies have used plasma polymerisation to form interlayers for the covalent grafting of antifouling PEG hydrogel layers,[69,70] including the plasma polymerisation of PEG-like tetra(ethylene glycol) dimethyl ether on to titanium surfaces for reduction of bacterial adhesion to dental implants. The antifouling effect of the coatings was studied by the fluorescent staining of bovine serum albumin (BSA), and showed a reduction in protein adsorption and cell adhesion without any significant cytotoxic effects.[71]

Other approaches of functionalising surfaces to repel/kill bacteria using plasma include deposited composite coatings embedding organic[72–74]/inorganic antibacterial agents[75,76], which have been reviewed in detail elsewhere.[77] A recent study attempting to address some of the problems of current antibiotic impregnation (including short life-span, narrow spectrum, and antibiotic resistance) has recently described the development of anti-biofilm

surfaces using bioactive, membrane-targeting antibacterial peptides.[78] Peptide-immobilised surfaces were prepared in a two-step process whereby material samples were first activated using argon plasma and then exposed to air to generate hydroperoxide reactive centers. Polymer grafted samples were immersed in lytic peptide solution, resulting in immobilisation via electrostatic interactions between negatively charged polymer chains and positively charged lytic peptides. Evaluation of anti-biofilm surfaces with *S. aureus* showed treated catheters to remain biofilm-free for up to 1 week under conditions of continuous cultivation, as well as increased stability of peptide films upon exposure to high concentrations of salt and biomacromolecules. Additionally, the low rate of antibiotic resistance and broad antibiotic spectrum makes lytic peptides excellent candidates in the future of CAUTI treatment.

Alternatives to the Foley Catheter

Despite numerous attempts to develop novel coatings and biomaterials to counteract the complications associated with long-term indwelling catheterisation, the Foley catheter remains the fundamental linchpin in the management of the dysfunctional bladder. Variations of the original catheter design,[79,80] as well as additive devices[81] to be used with the current clinical set up have been explored in order to circumvent the fundamental design flaws of the Foley catheter. Levering *et al*[82] have recently designed and optimised a urinary catheter prototype capable of on-demand biofilm removal *in situ*. Active surface deformation was used to detach biofilms from silicone substrates via utilisation of multi-inflation-lumen catheters with four intra-wall inflation lumens, allowing debridement of biofilms from the largely-inaccessible main drainage lumen. This extrudable catheter shaft design was able to supply sufficient strain around the intra-luminal perimeter to cause mixed-species biofilms of *P. mirabilis* and *E. coli* to debond from the material surface (Figure 2). Other attempts to redesign aspects of the urinary catheter away from Foley's original prototype include the trefoil design described by Sun *et al*.[83] The trefoil profile of the catheter proved successful in delaying the appearance of culturable organisms when tested in a small *in vivo* trial in rabbits. Variation in catheter design such as the trefoil profile may prove promising in the prevention of CAUTI. As well as the inherent advantages offered by the design itself, catheters such as this may also still be coated with passive or stimuli-responsive release coatings, thus giving the catheter a multifaceted approach to prevention of encrustation and blockage.

[Insert Figure 2]

Infection Detection Systems

A recently emerging approach to reduce associated patient morbidity and mortality is the detection of the crystalline biofilm formation in advance of catheter blockage. Thus, the catheter may be removed or replaced before the onset of the cascade of associated sequelae. Currently, there is no reliable way of preventing or accurately predicting when blockage may occur.[84] Patients whose catheters are known to block regularly may be scheduled regular catheter changes, however the unpredictability of catheter blockage remains, often resulting in emergency changes and the associated patient trauma and healthcare costs.[85]
In the case of long-term indwelling urinary catheters, freshly obtained urine from the catheterised bladder processed gives 50% more false negatives than urine evaluated in the same way from non-catheterised patients.[14]

A simple sensor described by Stickler *et al*[86] provides a colorimetric signal to warn of impending blockage. The sensor, located within the closed catheter drainage system, employs the dye bromothymol blue (BMB), which displays a pH-dependent colour change over the pH range of 6-8 (yellow to dark blue). Initial testing of cellulose acetate/BMB sensors *in vitro* was capable of giving a clear signal of *P. mirabilis* infection using pH as a proxy indicator. Further development of this system to enable larger scale manufacture employed a silicone-based composite strip design via combination of BMB with polydimethylsiloxanes and a hydrophobic filler.[87] The resultant strips of sensor material may be housed in a connector and placed within the junction between catheter and drainage bag, where they achieved the same yellow to blue colour change over the desired pH range approximately 19 hours in advance of blockage. Clinical evaluation of this system undertaken by Long *et al*[88] showed promising results, however the mean time between sensor colour change and catheter blockage was considerably longer in human trials than in the *in vitro* model resulting in an advanced warning of >19 days for the modified design.

Another dye-based diagnostic system, utilises a dual-layered polymeric system in the form of a catheter coating, whereupon a urinary colour change is resultant from an increase in urinary pH.[89] The system constitutes a lower hydrogel reservoir layer of ploy(vinyl alcohol) containing the self-quenching dye 5(6)-carboxyfluorescein, completely capped and sealed by an upper layer of the pH-responsive polymer poly(methyl methacrylate-*co*-methacrylic acid (EUDRAGITS 100®). Following infection by *P. mirabilis* and subsequent formation of alkaline urine, the outer layer of the coating swells to release the dye in its fluorescent form into the urine, giving a clear visual warning of impending blockage. *In vitro* analysis of the coatings using a clinically relevant artificial bladder demonstrated up to 12 hours advance warning of blockage, exclusively in the presence of urease-positive clinical isolates.

Surender *et al* have recently described a luminescent lanthanide pH-responsive probe capable of detecting the urease-mediated hydrolysis of urea in aqueous solution.[90] A series of photophysical titrations showed the Eu(III) chelate behave as an "*on-off*" luminescent switching probe, where the luminescence is quenched upon the conversion of urea to ammonia and carbon dioxide. Impregnation of water-permeable hydrogels with the lanthanide probe showed its potential to be used as a sensor in the field of CAUTI diagnosis (Figure 3A+B). Figure 3C shows the excitation spectra of the swollen Eu-based hydrogel, before and after the addition of urease. The Eu-centered emission was quenched by more than 85%, and the resultant colour change from bright red (*on)* to faint pink (*off)* was visible to the naked eye as well as under UV irradiation.

[Insert Figure 3]

Part II: Medical Approaches

Antibiotic-Based Approaches

Antibiotics have been central to the treatment of bacterial infections (including UTIs) since their discovery in 1928. Most cases of CAUTI are usually categorised as asymptomatic and thus do not require antibiotic prescription.[7] However, a number of antimicrobials (such as silver alloy and antibiotics) have been approved for use as topical antibacterial impregnated catheters for the prophylaxis of CAUTI.[7,91,92] These include nitrofurans, nitrofurazone and nitrofurantoin, which are known to have a broad spectrum of activity against Gram-negative and Gram-positive bacteria, and are less prone to causing bacterial resistance.[91] Nevertheless, these commercially available antimicrobial catheters still lack strong evidence to support their routine use in CAUTI management. Clinical trials assessing the performance of silver

alloy and nitrofurazone coated catheters in thousands of short-term catheterised patients showed no clinically relevant reduction in CAUTI incidence as compared to untreated catheters.[92] Furthermore, these catheter-treatments are vulnerable to *P. mirabilis* infections as silver-coated catheters were found to enhance crystalline biofilm formation, while nitrofurazone has little activity against *P. mirabilis* and lacked effective control of catheter blockage.[93,94] Full analysis of the efficacy of silver/antibiotic coated/impregnated catheters is beyond the scope of this review, but can be found elsewhere in the literature.[95–97]

The biocide triclosan has been tested previously for effective prevention of biofilm formation by various uropathogens including *P. mirabilis*, and prevention of catheter blockage.[98,99] The tests were conducted using representative *in vitro* models of the catheterized urinary tract, simulating a complete closed drainage system as used in clinical practice.[100] Catheter balloons were inflated with triclosan solution (3-10 g/L) diffusing into the bladder lumen in sufficient concentrations to prevent catheter encrustation in models inoculated with *P. mirabilis* only[99] and polymicrobial communities frequently containing *P. mirabilis*.[98] However, it was later revealed that extensive use of triclosan could potentiate an adverse therapeutic effect on *P. mirabilis*. Prolonged exposure of *P. mirabilis* to triclosan on agar plates resulted in selection of *P. mirabilis* mutants with reduced susceptibility to triclosan up to 300-fold, leading to catheter encrustation in models treated with triclosan.[101] Such vulnerability of individual antibiotic therapy, can be minimised by combination of antibiotics acting on different targets or using different mechanisms.

Fisher *et al.*[102] have reported impregnation of urinary catheters with a combination of rifampicin, sparfloxacin and triclosan (an antibiotic regimen chosen for its broad spectrum against CAUTI pathogens). Using a serial bacterial challenge within an *in vitro* model, antibiotic-impregnated catheter sections were inoculated with bacterial suspensions on a weekly basis, and antimicrobial activity was compared with two commercial silver-processed and nitrofural coated catheters. The novel coating demonstrated prevention of *Proteus mirabilis*, *Staphylococcus aureus* and *Escherichia coli* colonisation for 7–12 weeks, compared with 1–3 days for the commercially available antimicrobial catheters. No resistance was detected with any of the test bacterium during the 12 weeks of the flow challenge test. However, *P. mirabilis* isolates from catheters from week 8 showed a slight decrease in susceptibility to the antibiotics, suggesting mutational resistance still occurred. Further studies have demonstrated the efficacy of combined antibiotic formulations to control CAUTIs. For example azithromycin-ciprofloxacin-impregnated catheters were recently shown to avert *P. aeruginosa* bacterial colonization, prevent biofilm formation, and implant-mediated inflammation in a murine model.[103] Evidence from these studies show that antibiotic therapy is still relevant in CAUTIs. However the rise of antibacterial resistance, where potent antibiotics have been losing their efficacy over time requires alternatives to antibiotics.[104]

Alternatives to Antibiotics

Bacteriophage

Bacteriophage (phage) are likely to have high impact as alternatives to antibiotics for the control of CAUTIs. Phage are the natural viral predators of bacteria, and the most abundant biological entity on earth. Existing in nature as non-living particles,[105] they selectively infect bacteria and disrupt normal bacterial metabolism to self-replicate, and kill the bacterial host in the case of lytic phage. Lytic phage, that destroy bacterial cells releasing new phage particles are the most suitable for phage therapy and can be effective at a low phage dose.[106] Phage have been investigated for their antibacterial efficacy in a number of scientific disciplines, particularly within therapeutic delivery.[107]

Phage have showed great promise in a variety of formats including the development of antibacterial surfaces and biomaterials. In a study by Wang *et al*[108], active lytic bacterial viruses (bacteriophage) were immobilised onto polyhydroxyalkanoate (PHA) surfaces to form an infective antimicrobial surfaces. T4 bacteriophage (phage) immobilised on the surface after treatment with oxygen plasma retained their active conformation and orientation and hence were able to result in clear capture and infection of *E. coli* host cells. No evidence of phage binding was observed on untreated control films. (Figure 4).Such technology is potentially applicable to the treatment of CAUTI, since most polymers undergo similar oxidation during oxygen plasma treatment, thus the technique is widely transferrable to other polymeric substrates.

Recent studies have demonstrated the effect of phage against urinary pathogens using *in vitro* models simulating CAUTIs.[109–114] Pre-treating hydrogel-coated catheter sections with bacteriophages revealed an approximate 90% reduction in biofilm formation in *E. coli* and *P. mirabilis* species,[109] and 99.9% reduction in *P. aeruginosa* biofilms;[110] both studies employed a 24-h incubation static *in vitro* model. Using a two-species biofilm in a continuous-flow *in vitro* model, Lehman and Donlan[111] demonstrated the ability of bacteriophage cocktails coated within a hydrogel to reduce *P. aeruginosa* and *P. mirabilis* biofilms on catheters 99.9% to 99.8% over 48 hours. Reduction of biofilm formation and prevention of blockage of urinary catheters by *P. mirabilis* were also demonstrated,[113,114] using representative *in vitro* models of the catheterized urinary tract, simulating a complete closed drainage system as used in clinical practice.[100] When models simulating established infection of 10^{10} cells of *P. mirabilis*, were treated by a single dose of phage, directly added into the infected bladder model, the therapy significantly increased time taken for catheters to block (~3-fold). Phage therapy was able to completely eradicate early-stage infection consisting of 10^3 cells of *P. mirabilis,* when challenged with 10^{10} phage in bladder models. In these experiments, no viable bacterial cell was recovered from bladder model residual urine, whereas phage particles were still found to be present inside bladder models 8 days after model activation.[113]

[Insert Figure 4]

These studies suggest that high doses of phage delivered at an early stage of infection could lead to effective phage therapy, which is often hampered by development of phage-resistance reported in models with established infections.[111,113] Development of bacterial resistance to phage infection is usually caused by spontaneous mutations in host cell populations over time, or bacterial adaption in response to selective pressure exerted by the bacteriophage.[115] This leads to modifications in phage receptors, affecting phage-host interactions and efficiency of infection. One way to overcome phage resistance is the use of phage cocktails, where multiple phage are used in mixed dose therapy, resulting in broader antibacterial spectrum of activity.[116,117] Despite the great promise that phage therapy has shown throughout a number of scientific disciplines in recent years, the regulatory barriers are considerable and must be overcome before this can truly be seen as a viable treatment option.[107]

Drugs with Efflux Pump Activities

Efflux pump systems are transport proteins that bacteria use to extrude toxic agents, including antibiotics.[118] In their study investigating the genetic basis of biofilm formation in *P. mirabilis*, Holling *et al.*[119] demonstrated that efflux systems are essential in the formation of *P. mirabilis* crystalline biofilms. This was revealed by phenotypic characterisation of *P. mirabilis* mutants, which showed reduced ability to form crystalline biofilms and block catheters compared to the wild-type strain. Genetically, these mutants were disrupted in

the *Bcr/CflA* (a subfamily of efflux system).[119] This discovery led to further investigations for the potential for efflux pump inhibitors (EPIs) to control bacterial biofilm formation and catheter blockage by *P. mirabilis*. By screening several drugs already commonly used in human medicine, with potential EPI activity in other bacterial species,[120,121] Nzakizwanayo *et al.*[122] demonstrated the effect of fluoxetine and thioridazine to act as EPIs in *P. mirabilis*, and control biofilm formation and blockage of catheters. Fluoxetine is a selective serotonin re-uptake inhibitor (SSRI), marketed as Prozac, and thioridazine is an antipsychotic-drug. This study shows that repurposing of existing drugs with EPI activity as a novel approach to control catheter blockage.

Quorum-Sensing Inhibitors

The quorum-sensing (QS) system is a regulatory mechanism by which bacterial cells communicate via a chemical vocabulary (e.g. N-acyl homoserine lactones (AHLs) in Gram negative bacteria). Concentration of AHLs increases as the bacterial population grows, and upon reaching a threshold, binding to receptor molecules occurs. This binding acts as a transcriptional regulator on genes of the QS system and contributes to the production of virulence factors that aid biofilm formation.[123]Molecules and enzymes targeting this bacterial communication system have recently been investigated in the field of CAUTI as novel anti-biofilm agents. Jones *et al*[124] investigated two QS antagonists, tannic acid and *p*-nitrophenyl glycerol, finding that both were capable of controlling the crystalline biofilms of *P. mirabilis*. Anti-biofilm and anti-quorum sensing compounds from secondary metabolites of halophiles marine Streptomyces have recently been assessed by Younis *et al*.[125]Isolate sediment lake Iraq (sdLi) showed promising inhibition of *P. mirabilis* biofilm formation on urinary catheter, as well as attenuation of QS-dependent factors such as hemolysin activity, urease activity, pH and bacterial motility.

Coatings utilising the immobilisation of the enzyme acylase (from *Aspergillus melleus*) on biomedical grade polyurethane coatings via multipoint covalent immobilisation have shown to have potential in biofilm prevention via quorum sensing-disruption.[126] Coatings containing amylase were shown to enzymatically catalyse the hydrolysis of the quorum sensing molecules s N-butyryl-L-homoserine lactone (C4-LHL), N-hexanoyl-Lhomoserine lactone (C6-LHL), andN-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C12-LHL), and showed an approximately 60% reduction in biofilm formation by two clinical isolates of *P. aeruginosa*. A similar approach investigated by Ivanova *et al*.[127] where multi-layered coatings of acylase and α-amylase (able to degrade quorum sensing autoinducers and polysaccharides, respectively), deposited on urinary catheters via layer-by-layer techniques displayed quorum quenching and matrix-degrading abilities, delaying biofilm growth of relevant uropathogens by up to 7 days *in vivo*.

Enzymes

The preparation and antibacterial properties of an enzyme-embedded polycaprolactone (PCL)-based coating have been investigated by Dave *et al*.[128]The coating, impregnated with the antibiotic compound gentamicin sulphate (GS), utilises PCL itself as a substrate for the enzyme lipase, resulting in release of GS at a rate controlled by degradation of the PCL base. Modulation of enzyme concentration in PCL films allowed control of GS release from the coating from 16 to 33 days, also resulting in complete degradation of the polymer film so that no residual polymer substratum could serve as a base for possible microbial adherence post antibiotic release. The polymer showed antibacterial effects against three test isolates: *E. coli, S. aureus,* and *P. aeruginosa*, and displayed sustained release *in vitro* over a period of 60 hours when coated onto urinary catheters. The results suggest that the tunable, self-degrading coatings prove a promising candidate for use in urinary catheters, although enzyme biocompatibility within the bladder could prove problematic.

Enzymes have also been incorporated into catheter lubricants in order to investigate their potential in the field of CAUTI. Thallinger *et al*[129] examined the ability of cellobiose dehydrogenase (CDH) to produce hydrogen peroxide for antibiotic and antibiofilm functionalisation of urinary catheters. Biofilm formation of silicone films was prevented by 1 mM CDH (recombinantly produced from *Myriococcum thermophilum*), and the growth of many common uropathogens, including *P. mirabilis*, was inhibited. The aforementioned antibiofilm activities were found to be strongly enhanced when used in the presence of α-linked glycosides, as the consequent hydrolysis of polysaccharides increases the number of terminal reducing sugars as substrates for CDH as well as causing destabilisation of the biofilm.

Part III: Stimuli-Responsive Approaches

Alongside the current engineering and passive release strategies aimed at preventing catheter blockage, additional research has focused on stimuli-responsive systems. Such systems rely on an external stimulus (either directly or indirectly associated with the onset of infection) in order to facilitate diagnosis or treatment. Direct indicators of infection include specific biomarkers produced by pathogenic bacteria (such as enzymes or toxins), whilst a change in the local environment may indirectly confirm infection via bacterial or immune activity (e.g. a change in pH or temperature). Stimuli-responsive materials retain their diagnostic or therapeutic potential until an endogenous factor initiates a burst response of the cargo contained within. This may manifest as a change in volume or structure of the material (in the case of polymeric or hydrogel formulations), or as a result of bond cleavage of anchored moieties.[39] The ability to control both the release kinetics and the delivery location of a diagnostic or therapeutic agent offers certain advantages over conventional passive release systems including: protection of the often sensitive cargo from harsh external conditions and avoidance of any unnecessary or sub-lethal exposure, thus limiting any subsequent evolution of bacterial resistance. The controlled release concept is especially attractive in the treatment of CAUTI owing to the time-dependent delivery of the therapeutic cargo. In addition to the aforementioned precautions, premature administration of a therapeutic agent to the bladder will inevitably result in timely elution, rendering the system ineffective in successfully clearing a progressive infection.

The urease-catalysed conversion of urea into ammonia by certain urinary pathogens (in particular *P. mirabilis*), and the resultant increase in urine pH has been exploited in the development of a number of pH-responsive catheter coatings. The pH-responsive polymer Eudragit S100® (poly (methylmethacrylate-co-methacrylic acid)) has been developed in order to deliver bacteriophage from coated catheters in order to delay blockage. Prior to any elevation in urine pH, *P. mirabilis* bacteriophage contained within the PVA reservoir layer are unable to elicit any bactericidal effects. However, upon an infection-associated increase in urine pH and the subsequent dissolution of the Eudragit layer, the phage are able to diffuse into the bladder, reducing bacterial load by up to 6-log within 2 hours. Overall, this resulted in doubling the time to blockage (26 hours compared to 13 hours for the uncoated catheters), ultimately extending the catheter lifetime by 100%.[114]

An alternative strategy in the development of pH-responsive catheter coatings utilises the solubility of specific drug formulations in order to modulate their release from polymeric hydrogels. Nalidixic acid (a pH sensitive antimicrobial quinolone) has been successfully loaded into polymeric films of poly(2-Hydroxyethylmethacrylate) co-polymerised with methyl methacrylate. Owing to the presence of an ionisable carboxyl group within the quinolone structure, the mechanism of pH-dependent drug release relies on the affinity of the

ionised drug (at alkaline pH) towards aqueous media (urine), resulting in diffusion out of the carrier matrix and into the surrounding area. As opposed to conventional polymeric triggered release systems, this formulation does not rely on a structural change in the polymeric carrier in order to facilitate antimicrobial expulsion, rather the chemical properties of the antimicrobial itself govern the rate of drug release. Utilising a hydrophobic polymer, the release kinetics of the drug were controlled in order to provide sustained zero-order drug diffusion over the course of weeks, as oppose to hours as reported with more hydrophilic polymers.[130] Moreover, a series of polymerisable ester naphthyridinone conjugates have been synthesised with varying chain lengths. The base-catalysed hydrolysis of the ester bond linking the nalidixic acid to the vinyl spacer arm determines the rate of pH-dependent drug release when anchored to polymeric biomaterials.[131] Further studies have developed this concept using the aforementioned vinyl-functionalised nalidixic acid and crosslinked 2-hydroxyethyl methacrylate hydrogels, forming antimicrobial pro-drug matrices. *In-vitro* results exhibited negligible drug release at physiological pH, while the triggered, prolonged release of nalidixic acid was up to 20-fold faster and approximated 80% after 6 weeks at pH 10.[132]

In contrast to utilising a change in the external environment, specific bacterial biomarkers have been exploited in order to induce the release of antibacterial agents via disassembly of polycationic nanospheres. Aminocellulose (AC), an effective antimicrobial agent owing to its propensity towards bacterial membrane disruption, has been formulated into nanospheres and incorporated into a multi-layer coating capable of preventing *P. aeruginosa* biofilm formation. The formulation comprises AC nanospheres coated with hyaluronic acid, the outer layer is degraded by pyocyanin (a redox active virulence factor produced by *P. aeruginosa*) thus liberating the antimicrobial components within. Planktonic bacterial growth was reduced by 70% after 2 hours ($p < 0.001$), whereas under dynamic conditions (bladder model experiments) effective catheter biofilm prevention was confirmed via fluorescence microscopy as shown in Figure 5.[133]

[Insert Figure 5]

Concluding Remarks and Future Perspective

Whilst it is widely acknowledged that the clinical, social and economic burden associated with the use of the Foley catheter for long-term indwelling catheterisation has gained notoriety in recent years, it is also true to say that regulatory authorities, relevant industrial and commercial companies as well as the scientific community has thus far failed to seek a comprehensive solution. This is likely owing to the fact that in order to achieve such an extensive and complex scientific undertaking, the nature of the necessary research is inherently multidisciplinary. The marriage of broad and often tenuously linked scientific disciplines must occur in order to truly overcome the problem of encrustation and blockage of Foley catheters. Indeed, it is the responsibility of the commercial companies and regulatory authorities to positively encourage innovation for an application where existing devices are so clearly inadequate. Taking into account the research presented in this review, future attempts to improve the urinary catheter system must involve the marriage of chemical, biological and engineering advancements, whilst still maintaining the assets of the original design. It is necessary for research funders, healthcare services and governing bodies to stimulate interdisciplinary research amongst the scientific, engineering, industrial and clinical communities to meet and overcome the challenge.

Moreover, passive release of antimicrobial cargos should not be considered a panacea or universally effective strategy as previously regarded. Rather, they should be considered part of a concerted effort to control known risk factors of CAUTI, and the basis of more advanced and microbiologically fool proof methods, such as stimuli-responsive release. Such 'smart' coatings provide the basis of a multifaceted solution in which the properties and local concentration of the antimicrobial agent are maintained whilst limiting systemic exposure, thus taking into consideration the challenge of introducing such species into the catheterised bladder. Despite recent advances in this field, several key challenges must be overcome in order for triggered release coatings to become a truly valued tool in the CAUTI-preventative arsenal, including long term stability, maintenance of catheter functionality and ease of commercial manufacture.

In conclusion, the review has identified the current advances within the fields of medicine and biomedical engineering, which must now be used in combination in order to maximise their efficacy and broaden their field of application. Despite the large amount of reported approaches throughout the literature, few platforms have progressed to clinical studies, and even fewer to clinical practice. The lack of translational success can be attributed in part to the inherent complexity of the problem and the necessity of the multidisciplinary approach necessary to overcome it. Objective evaluations of coating stability, specific to the challenge of CAUTI have often been similarly overlooked. Specifically structured research is therefore paramount to develop validation methods for technologies such as stimuli responsive coatings, such that clinical efficacy may be effectively extrapolated. Overcoming these challenges will require collaborative effort from those working across a wide variety of disciplines (in particular the field of biomedical science), but success will offer ample opportunity for innovation, as well as a long-overdue solution to a complex clinical problem.

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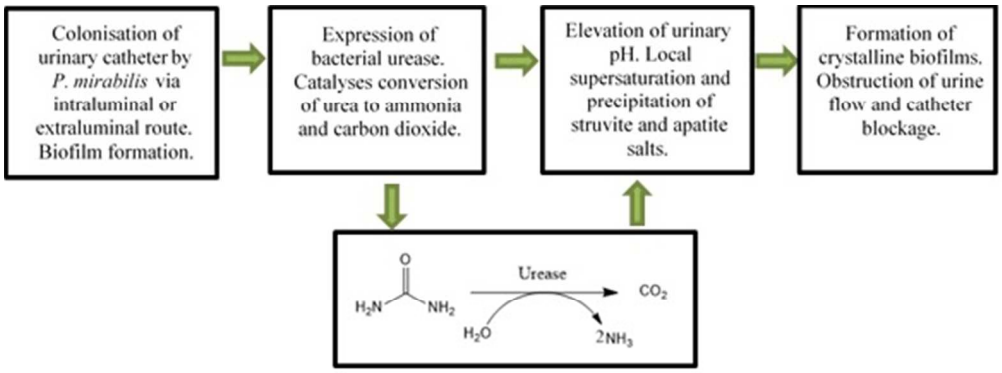

Figure 1: Flowchart showing the sequential encrustation and blockage of an indwelling urinary catheter following infection by *Proteus mirabilis*.

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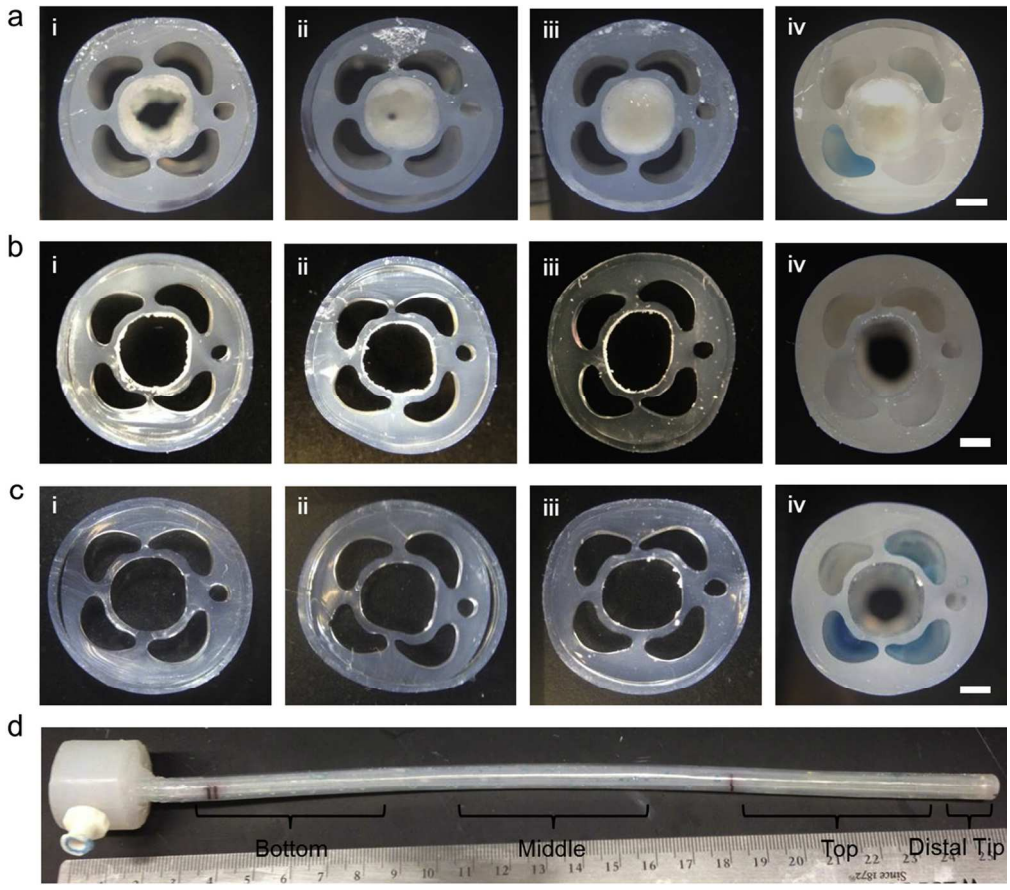

Figure 2: Multi-inflation-lumen catheters able to cause debridement of multispecies *P. mirabilis* and *E. coli* biofilms in situ. Cross sectional images of A) control catheter (no inflation), B) first inflation after 30 hours of biofilm growth, C) second inflation after 24 hours biofilm regrowth. D) Sections from which the cross sections were obtained: i) bottom, ii) middle, iii) top, and iv) distal tip. Scale bars indicate 1 mm. Reprinted from [82]. © 2016, with permission from Elsevier.

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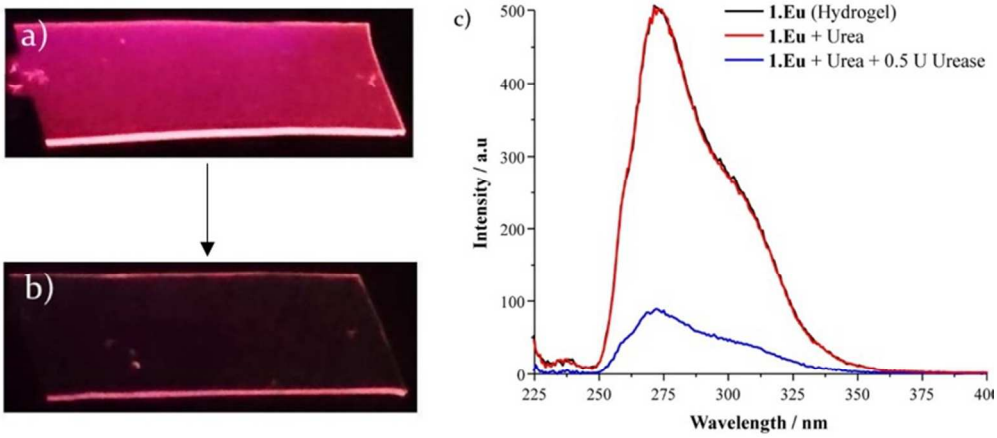

Figure 3: Assessment of a hydrogel-impregnated supramolecular Eu(III)-based pH-responsive "on-off" sensor for CAUTI. A) "on" (before addition of urease), B) "off" (200 minutes after addition of urease) states of the hydrogel, when irradiated at λmax = 254 nm. C) Excitation spectra of the swollen Eu-based hydrogel, before and 200 minutes after the addition of urease (0.5 U), measured in an aqueous solution of urea (2.3 x10-3 M) at 295 K. Adapted with permission from [90]. © 2016 American Chemical Society.

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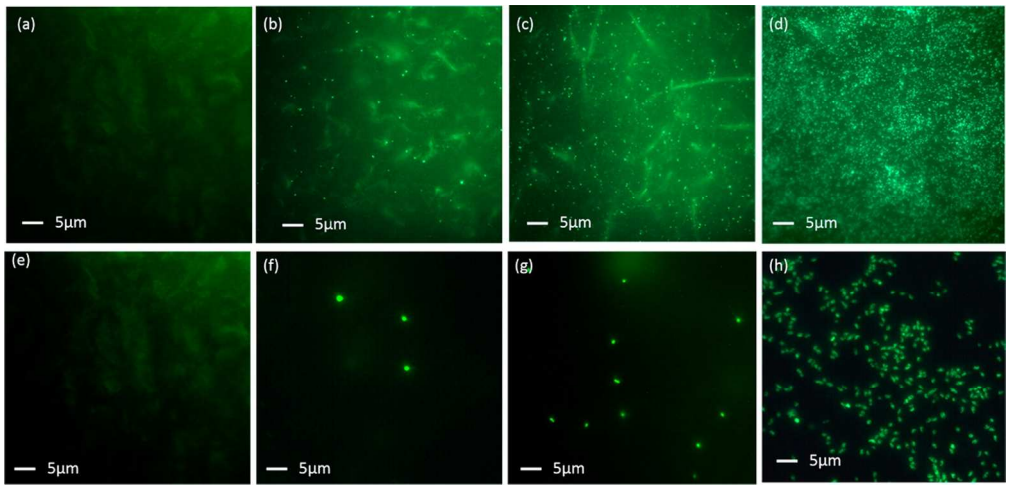

Figure 4: Fluorescence microscopy images of PHA surfaces treated with oxygen plasma and incubated with carrying concentrations (plaque forming units per millilitre (PFU/ml)) of T4 bacteriophage. Top row: characterisation of immobilised bacteriophage on PHA. Bottom row: Stained *E. coli* host cells in stationary phase, captured on surfaces with immobilised bacteriophage. A, E) Control films (no bacteriophage). B,F) Films prepared with 2 x 108 PFU/ml. C,G) Films prepared with 2 x 109 PFU/ml. D,H) Films prepared with 2 x 1010 PFU/ml. Both phage and bacterial cells were stained using SYBR green, 1 hour post-immobilisation and prior to exposure, respectively. Reprinted with permission from [108]. © 2016 American Chemical Society.

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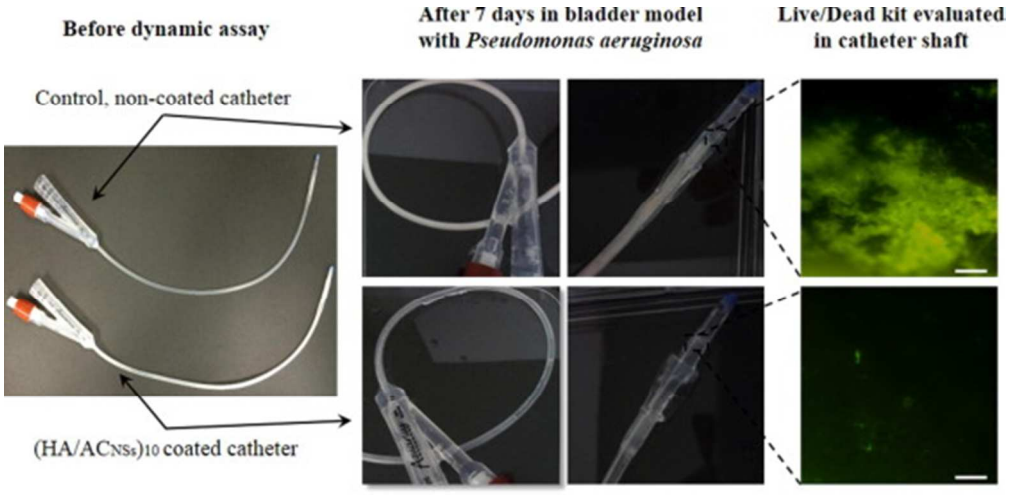

Figure 5: Effect of hyaluronic acid coated AC nanospheres (HA/ACNSS) on *P.aeruginosa* biofilm formation within the shaft of a foley catheter after 7 hours incubation (bottom), compared to the uncoated control catheter (top). Fluorescent microscopy images are 20 x mag with scale bars set at 100 μm. Reprinted from [133]. © 2016, with permission from Elsevier.

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