Biosensors and Bioelectronics 81 (2016) 166–172

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

An *in-situ* infection detection sensor coating for urinary catheters

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ARTICLE INFO

Article history:
Received 14 January 2016
Received in revised form
22 February 2016
Accepted 23 February 2016
Available online 26 February 2016

Keywords:
Catheter-associated urinary tract infections
Proteus mirabilis
Carboxyfluorescein
Hydrogels
Sensor

ABSTRACT

We describe a novel infection-responsive coating for urinary catheters that provides a clear visual early warning of *Proteus mirabilis* infection and subsequent blockage. The crystalline biofilms of *P. mirabilis* can cause serious complications for patients undergoing long-term bladder catheterisation. Healthy urine is around pH 6, bacterial urease increases urine pH leading to the precipitation of calcium and magnesium deposits from the urine, resulting in dense crystalline biofilms on the catheter surface that blocks urine flow. The coating is a dual layered system in which the lower poly(vinyl alcohol) layer contains the self-quenching dye carboxyfluorescein. This is capped by an upper layer of the pH responsive polymer poly (methyl methacrylate-*co*-methacrylic acid) (Eudragit S100®). Elevation of urinary pH (> pH 7) dissolves the Eudragit layer, releasing the dye to provide a clear visual warning of impending blockage. Evaluation of prototype coatings using a clinically relevant *in vitro* bladder model system demonstrated that coatings provide up to 12 h advanced warning of blockage, and are stable both in the absence of infection, and in the presence of species that do not cause catheter blockage. At the present time, there are no effective methods to control these infections or provide warning of impending catheter blockage.

1. Introduction

It has been estimated that ~100 million indwelling urinary catheters (IUC) are sold annually worldwide (Saint et al., 2000). In the US alone, ~30 million urinary catheters are fitted each year, making IUCs by far the most commonly deployed medical device, with levels of use far outstripping other common devices such as central venous catheters or fracture fixation devices (Darouiche, 2001). Although in many cases the use of IUCs can benefit patients and greatly aid treatment and recovery, these devices undermine the innate barriers to bacterial colonisation naturally present in the urinary tract, thus predisposing patients to infection by uropathogenic bacteria (Stickler and Zimakoff, 1994; Stickler, 2014). Given the widespread use of these devices, it is perhaps unsurprising that catheter associated urinary tract infections (CAUTIs) are currently among the most common nosocomial infections in many healthcare settings (Stickler, 2014; Jacobsen et al., 2008; Tambyah, 2004; Hooton et al., 2010; Getliffe and Newton, 2006). CAUTIs pose a serious risk to patient welfare and a significant financial burden to health service providers, exemplified by estimated costs of up to ~£123 million per annum in the UK, and $424–451 million per annum in the USA (Jacobsen et al., 2008; Ploughman et al., 1999).

The problem of CAUTI is particularly pronounced in patients who are managed long-term with urethral catheterisation, where IUC are in place for weeks or months at a time. This includes many elderly individuals and those with spinal cord injuries, in whom urethral catheterisation is often used to manage incontinence in a community care setting (Buckley and Lapitan, 2009; Sørbye et al., 2005; McNulty et al., 2003; Getliffe, 1994). One of the most problematic and severe complications arising from CAUTI in this group is the encrustation and blockage of catheters, which may be experienced by up to 50% of patients undergoing long-term urethral catheterisation (Getliffe, 1994). Encrustation and blockage is almost exclusively due to infection by *Proteus mirabilis*, which is isolated from up to 45% of CAUTIs (Stickler, 2014, 2008; Stickler et al., 1993; Mobley, 1996). Following the initial colonisation of the urinary tract, *P. mirabilis* develops extensive biofilm communities on catheter surfaces, characterised by aggregations of cells embedded in a dense exopolymeric matrix (Stickler 2014, 2008; Donlan, 2002). Biofilms are intrinsically resistant to immune clearance, antimicrobial agents, and environmental factors, hence treatment of infections involving biofilms is in itself a major medical problem (Stickler, 2014, 2008; Donlan, 2002). Concurrent with biofilm development, *P. mirabilis* expresses a highly potent urease enzyme during growth in urine, allowing exploitation of urea as a nitrogen source (Griffith et al., 1976). The activity of this

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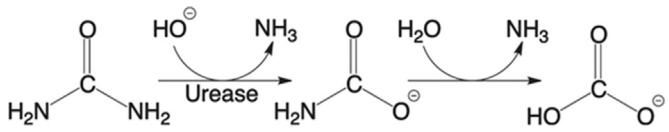
http://dx.doi.org/10.1016/j.bios.2016.02.059



Scheme 1. Urease-catalyserd hydrolysis of urea as a nitrogen source for *P. mirabilis*.

urease enzyme (Scheme 1) generates ammonia, elevating urinary pH, and leading to the precipitation of calcium phosphate and magnesium–ammonium phosphate from urine to form crystals of carbonate apatite [$Ca_{10}(PO_4)_6CO_3$], and struvite ($MgNH_4PO_4 \cdot 6H_2O$), respectively (Stickler et al., 1993; Griffith et al., 1976; Holling et al., 2014a, 2014b). These crystals subsequently become incorporated into the developing biofilms, which further stabilises and enhances their growth (Stickler et al., 1993; Holling et al., 2014a, 2014b). Through these processes, extensive abrasive crystalline biofilm structures are formed which encrust catheter surfaces and eventually block urine flow.

Catheter blockage causes painful retention of urine within the bladder, and subsequent vesico-ureteric reflux of infected urine to the kidneys (Stickler, 2014; 2008). If blockage is not detected before this occurs, patients suffer episodes of severe kidney infection and septicaemia (Stickler, 2014; 2008). Unfortunately, as the majority of long-term catheterised patients are cared for in the community, where constant clinical surveillance is not available, blockage typically remains unnoticed until life threatening consequences arise, and hospital treatment is required Stickler (2014). Although a range of catheters impregnated with antimicrobials are widely available, their use in controlling infection even during short-term catheterisation (<7 days) remains questionable (Pickard et al., 2012; Morris et al., 1997; Morgan et al. 2009).

Currently, two such "infection control" catheters are available in the United Kingdom National Health Service: a silver alloy-coated latex catheter, and a nitrofurazone-impregnated silicone plastic catheter. However, recent meta-analysis of clinical studies has shown that silver alloy-coated catheters were unable to significantly reduce symptomatic CAUTI compared with standard catheters, and were considered not to be cost-effective. Nitrofurazone-impregnated catheters showed borderline clinical benefit, however, any benefit was offset by a marked increase in patient discomfort, and concerns regarding the indiscriminate use of antibiotics (Pickard et al., 2012). Furthermore, all currently available catheter types remain susceptible to encrustation, including those with antimicrobial coatings (Stickler, 2014; Pickard et al., 2012; Morris et al., 1997; Morgan et al., 2009). As such, available infection control catheters provide little or no benefit for long-term catherised patients where the onset of bacteriuria (growth of bacteria in urine) is considered to be almost inevitable (Stickler, 2014; Jacobsen et al., 2008; Kunin, 1997). While bacteriuria and the majority of CAUTI are asymptomatic and typically not treated, the serious complications induced by blockage highlight the need for strategies to better identify and manage *P. mirabilis* infections, with the ultimate goal of warning patients and carers that blockage may be imminent (Stickler, 2014).

The concept of using urinary pH elevation to provide infection responsive drug release was explored by Irwin et al. (2013), who successfully achieved controlled release of nalidixic acid from poly (2-hydroxyethylmethacrylate (p(HEMA)) hydrogels. Here, a novel "early warning" system is described, which is designed to alert patients and carers of forthcoming catheter blockage. The system takes the form of an infection-responsive surface coating, compatible with existing catheter designs, able to provide a visual warning of *P. mirabilis* infection prior to encrustation and blockage. The coating consists of a dual-layered polymeric architecture, in which a lower layer of hydrogel (poly (vinyl-alcohol)) is employed to encapsulate the self-quenching dye 5(6)-carboxyfluorescein, at concentrations sufficient to inhibit fluorescence. This lower layer is capped and sealed by an upper pH-sensitive 'trigger' layer, ensuring no dye release while this is in place (Fig. 1). The 'trigger' layer is composed of EUDRAGIT®S 100 (an anionic co-polymer of methacrylic acid and methyl methacrylate). Elevation of urinary pH upon *P. mirabilis* infection (*via* the urease-catalysed hydrolysis of urea) causes dissolution of the upper EUDRAGIT®S 100 layer, releasing the carboxyfluorescein contained in the lower hydrogel matrix to provide a clear visual signal throughout the catheter drainage system that blockage is imminent, and intervention is required.

2. Experimental section

2.1. Silanisation of Foley catheters

In order to coat the hydrophobic silicone catheter with PVA, it was necessary to modify the surface with a hydrophilic moiety. In this case, a silane (3-Aminopropyl triethoxysilane) (APTES) introduced ethoxy groups on the catheter surface. The method for hydrophilisation of catheter surfaces via silanisation of the silicone surface was modified from Gencer et al. (2012). Catheters were washed in 1:1 mixture of ammonia (33% v/v) and hydrogen peroxide (30% v/v) for 10 min with constant shaking, then rinsed with deionized water and dried under nitrogen. The catheter was then placed in APTES (1% V/V) dissolved in dry N,N-Dimethylformamide (DMF) for 16 h. The surface-modified catheters were subsequently washed with DMF and dried under nitrogen. Water contact angle measurements were made to ensure the hydrophilic nature of the surface.

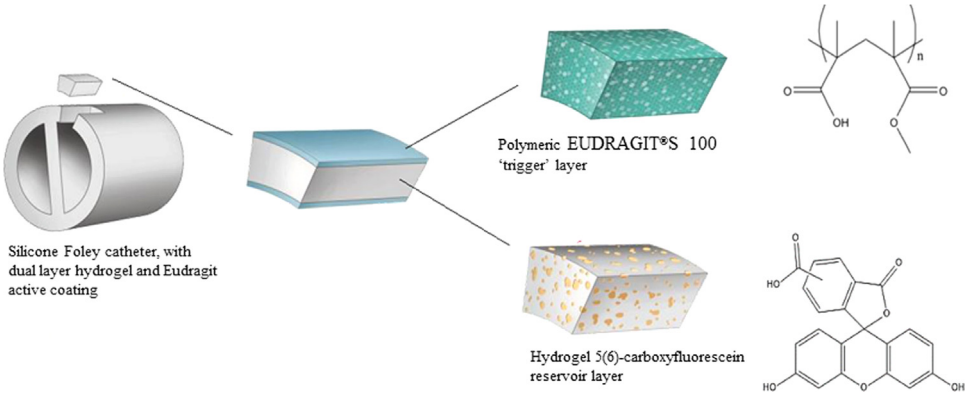


Fig. 1. Schematic illustration of dual-layered polymeric architecture for pH-triggered release of 5(6)-carboxyfluorescein.