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The potential of photo-deposited silver coatings on Foley catheters to prevent urinary tract infections

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ABSTRACT

Catheter-associated urinary tract infection (CAUTI) represents one of the most common causes of morbidity and mortality. The resistance demonstrated by many microorganisms to conventional antibiotic therapies and the increasing health-care costs have recently encouraged the definition of alternative preventive strategies, which can have a positive effect in the management of infections. Antimicrobial urinary catheters have been developed through the photo-chemical deposition of silver coatings on the external and luminal surfaces. The substrates are exposed to ultraviolet radiation after impregnation into a silver-based solution, thus inducing the *in situ* synthesis of silver particles. The effect of the surface treatment on the material was investigated through scanning electron microscopy (SEM) and silver ion release measurements. The ability of microorganisms commonly associated with urinary tract infections was investigated in terms of bacterial viability, proliferation and biofilm development, using *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* as target organisms. The silver coatings demonstrated good distribution of silver particles to the substrate, and proved an effective antibacterial capability in simulated biological conditions. The low values of silver ion release demonstrated the optimum adhesion of the coating. The results indicated a good potential of silver-based antimicrobial materials for prevention of catheter-associated urinary tract infection.

1. Introduction

The urinary tract is a sterile environment in healthy people, and represents one of the most susceptible sites for bacterial infections in the human body [1]. Urinary tract infections (UTIs) are one of the most common nosocomial infections, more commonly experienced by women than men; and approximately 80% of UTIs are associated with the presence of an indwelling urinary catheter [1–3]. Catheterisation is one of the most common modern medical procedures, where the insertion of a urinary catheter bypasses the normal host defences facilitating the entry of pathogens into the bladder [2]. The bacterial pathogens most commonly associated with catheter-associated urinary tract infections (CAUTIs) are *Escherichia coli* and *Proteus mirabilis* [4]. An infection associated with *Proteus mirabilis* frequently leads to blockage of catheters because of biomineralisation and formation of a crystalline biofilm through the bioconversion of urinary ammonium and other salts, causing obstruction of catheter lumen and kidney infection and septicaemia [4–6]. *Klebsiella pneumoniae* is a gastro-intestinal bacterium and an opportunistic pathogen associated with septicaemia, pneumonia, urinary tract infections, and meningitis, and can persist within the urinary tract despite appropriate antibiotic treatments [7]. Colonisation by *Pseudomonas aeruginosa* and *Candida* spp. may also be observed in patients undergoing prolonged catheterizations [4]. After long-term use, monobacterial biofilm formation has been observed to occur, sometimes with multi-drug resistant organisms, on many urinary devices [8]. Bacteria have been shown to colonise the catheter surface and form a biofilm on both the internal (intraluminal) and external (extraluminal) surfaces of the catheter, suggesting that intervention strategy must address the issue of reducing or preventing bacterial colonisation on both surfaces [9,10]. Prolonged catheterisation is the most important risk factor associated with CAUTI, and the formation of biofilm plays a central role in the pathogenesis of CAUTIs [11]. Biofilms are heterogeneous structures composed of an accumulation of microorganisms and their extracellular products forming a structured community on a surface [12,13]. These biological structures are difficult to eradicate because of biofilm-specific mechanisms of tolerance and drug resistance, with the cells and associated materials able to persist on bladder epithelium despite the removal of the catheters [13,14]. The increasing reports of

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antibiotic resistance amongst members of the normal human microbiota has been shown to exacerbate the recalcitrance of bacterial biofilms on medical devices, limiting available therapeutic options. This is a serious medical and public health issue that requires the application of novel approaches to prevent biofilm growth on indwelling catheters [15]. An alternative approach is the incorporation of these antimicrobial agents into biomaterials used in catheter production, thus developing a catheter surface that could inhibit the bacterial growth [16]. The lack of innovative antimicrobials released on to the pharmaceutical market in recent years has led to considerable concern from the World Health Organization (WHO). In 2014, the WHO global report on surveillance of antimicrobial resistance outlined the possibility of mortality associated to common infections such as those induced by *Escherichia coli* [17]. Progress and implementation in infection control programs are necessary and require technical advances in catheter materials for prevention of biofilm formation [18]. A variety of strategies, such as the replacement of contaminated catheters, novel materials for catheter production and surface functionalization with antibacterial agents have been suggested [4,15], including rifampicin, sparfloxacin and triclosan have been proposed for impregnation of urinary catheters [4]. Currently, the use of silver in biomedical field is increasing because of its broad-spectrum antimicrobial activity demonstrated against multi-drug resistant bacteria, fungi and viruses [19,20]. At nanometric size, silver is extremely active against microorganisms due to the high surface to volume ratio [21]. In this work, silver coatings have been deposited on urinary catheters on the inner and outer surfaces through a technique based on the photo-reduction of a silver precursor. Scanning electron microscopy (SEM) and silver ion release measurements through inductively coupled plasma mass spectrometry (ICP-MS) were performed to investigate the properties of the silver coating and to determine the effect of the silver treatment of the material. The antimicrobial activity has been evaluated against the most common microorganisms responsible for CAUTI through specific microbiological characterizations aiming to simulate the biological environment of the devices.

2. Materials and methods

2.1. Preparation of samples

The focus of this study was silicone-coated Foley latex urinary catheters (18 French) designed for short-term use only (less than 30 days). They have been deposited with silver coatings by adopting a technology based on the photochemical deposition of silver nanoparticles [22]. The method involves depositing a silver solution on the surface of the material and inducing the *in situ* synthesis and deposition of silver particles through the UV irradiation of the wet substrate. The silver solution adopted for this specific treatment has been prepared by dissolving silver nitrate (2 w/v%) in methanol (98 v/v%) under magnetic stirring at room temperature. Pieces of catheters were impregnated with the silver solution through dip coating and exposed to a UV lamp (Jelosil) with an emission peak at 365 nm for 10 min. During the UV treatment, the samples were moved to allow the irradiation on the inner surface. Next, the catheters were washed carefully in order to remove any trace of unreacted silver salt and, then, they have been characterized.

2.2. SEM-EDX analysis

Scanning electron microscopy (SEM) (Zeiss EVO) was performed by means of a backscattering detector on sections of untreated and silver treated catheters to investigate the presence and distribution of silver particles on the outer and luminal surfaces of the device. Additional Energy Dispersive X-ray Spectroscopy EDX (Bruker) was carried out to determine the surface composition and the presence of silver on both the surfaces of the device.

2.3. Silver ion release

The release of silver ions from the silver-treated substrates in artificial urine was analysed through ICP-MS (Thermofisher iCAP-Q) at the same time points selected for the microbiological characterization. The samples (length 1 cm, average weight 0.2813 g) were incubated at 37 °C in 3 mL of artificial urine medium for 1, 3, 7, 10 and 14 days. At each time point, the samples of catheters were removed from the tubes and the artificial urine was stored at 4 °C for analysis, and were conditioned at room temperature for 1 h. Transition elements calibration standard CCS-6 and 1, 10, 50 and 100 ppb concentrations were selected for calibration. Artificial urine was used as the analytical matrix (see below for constitution), and 1% HNO_3 was used for dilutions.

2.4. Microbiological characterization

All microbiological media was purchased from Oxoid Ltd. (UK), and all chemicals were purchased from Fisher Scientific (UK). All experiments were performed in triplicate. The bacterial strains used in this work were *Escherichia coli* NCIMB 8545, *Klebsiella pneumoniae* NCTC 11228 and *Proteus mirabilis* NCTC 11938.

2.4.1. Artificial urine medium

Artificial urine (AU) was adapted in-house upon the original work of Stickler et al. [23]. The final constitution of the stock media was as follows, for 100 mL total volume: 2.3 g sodium disulphate, 0.65 g magnesium chloride, 4.6 g trisodium citrate, 0.02 g sodium oxalate, 2.8 g potassium dihydrogen orthophosphate, 1.6 g potassium chloride, 1 g ammonium chloride, 5 g gelatin, and 1 g tryptone soy broth. Reverse osmosis water was used to constitute the medium, which was subsequently autoclaved at 121 °C for 15 min. A solution of urea and calcium chloride was also constituted; comprising 25 g of urea and 0.65 g of calcium chloride in 400 mL of reverse osmosis water was also added. This was filter sterilized using a 0.2 μm syringe filter in a Class II microbiology hood. Next, 92 mL of the stock medium was added to 8 mL of the urea/calcium chloride solution to make the artificial urine medium. These 100 mL aliquots were prepared aseptically, sealed and stored until needed.

2.4.2. Sample preparation

Samples of untreated and treated Foley catheters were cut in to 1 cm sections using a sterile scalpel within a Class II microbiology hood. These samples were sterilized by exposure to UV irradiation at 250 nm on each side for 30 min, and then sealed in new sterile plastic bags to minimize the risk of contamination. Sterility was determined by the inoculation of replicate samples in to tryptone soya broth medium. Macroscopic growth was checked at 24, 48 and 72 h visually, and at these time points 100 μL aliquots were spread on to tryptone soya agar plates (in triplicate) and incubated for 72 h at 37 °C, with inspection for colony growth at 24, 48 and 72 h, respectively.

2.4.3. Biofilm growth

Bacterial biofilms were cultivated using an adaptation of the method of Cooper and Hanlon [24]. Briefly, 1 cm^2 catheter sections were immersed in 3 mL of sterile artificial urine (AU) medium in six-well plates and inoculated with 100 μL of a 1×10^6 CFU/mL of each bacterial strain, respectively. These were left to sediment at room temperature for 1 h, after which, the sections were transferred into new six-well plates containing 3 mL phosphate buffered saline (PBS), and placed onto an orbital shaker for 15 min at 120 rpm (Stuart Scientific, UK) so as to dislodge any non-adherent cells. After this time, the sections were incubated at 37 °C. At time points 3, 7, 10 and 14 days, the sections to be investigated for biofilm growth were removed and placed in fresh six-well plates containing 3 mL PBS on an orbital shaker for 15 min at 120 rpm to remove any non-adherent cells. After this time, the samples were placed into 10 mL PBS containing glass balls, and vortexed for 1 min. These samples