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Silver doped perfluoropolyether-urethane coatings: Antibacterial activity and surface analysis

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ABSTRACT

The colonisation of clinical and industrial surfaces with pathogenic microorganisms has prompted increased research into the development of effective antibacterial and antifouling coatings. There is evidence that implanted biomedical surfaces coated with metallic silver can be inactivated by physiological fluids, thus reducing the bioactivity of the coating. In this work, we report the biofilm inhibition of *Staphylococcus epidermidis* using a room temperature processed silver doped perfluoropolyether-urethane coating. The release of silver ions from these fluoropolymers over a six-day period inhibited bacterial encrustation – as observed by scanning electron microscopy (SEM). X-ray photoelectron spectroscopy (XPS) analysis indicated differences in carbon, fluorine and sodium surface composition between silver doped and undoped fluoropolymers after exposure to nutrient rich media. These silver doped perfluoropolyether coatings also exhibited antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*; suggesting potential use in preventing transmission of pathogenic and opportunistic microbes on environmental surfaces in healthcare facilities. The broad-spectrum antibacterial activity of these silver release coatings may be exploited on biomaterials surfaces to combat the development of resistant Gram-negative *Enterobacteriaceae* that can occur during prophylactic treatment for urinary tract infections.

1. Introduction

Bacteria such as *Staphylococcus aureus* have shown adaptibility to changing environments in the healthcare setting, but also more recently in the wider community [1–3]. Opportunistic pathogens such as *S. aureus* and *Pseudomonas aeruginosa*, along with other bacteria, can develop resistance through an elaborate array of mechanisms. These include modification of the target protein or binding site, enzymatic destruction of antibacterial agents, active efflux of drugs from the bacterial cell or by acquisition of genetic material from other resistant strains [4,5]. The emergence of bacterial resistance often occurs shortly after the introduction of a new antibacterial agent. An increase in resistance can be observed by an elevated minimum inhibitory concentration (MIC) of the antibacterial agent [6]. This can be exemplified by the rapid emergence of *Enterococcus faecium* resistance to anti-VRE/MRSA antibiotics, such as linezolid [7].

The reduction in the number of available antibacterial therapies for the treatment of multi-drug resistant pathogens, has resulted in a reversion to older antimicrobial agents, such as silver [8]. In medical terms, silver is the antimicrobial metal best known for its broad-spectrum activity against intractable infectious microorganisms, including antibiotic resistant strains [8]. The multiple modes of action of silver include binding with cell DNA, inhibiting enzymes that mediate respiration and reacting with sensitive thiol groups on bacterial proteins – thereby destroying the normal biological activity of the protein [9]. The antimicrobial properties of nanosilver loaded filter paper against *Escherichia coli* have been recently studied [10]. The use of medical device coatings as release or delivery vehicles for these biocidal silver ions has received significant attention recently. Silver ion release from sol–gel coatings has demonstrated antibacterial activity against *S. aureus* and *E. coli* [11–12]. Nanosilver coated fabric inhibited the growth of the Gram-negative bacterium *E. coli* [13]. Silver coated PTFE surfaces have shown antibacterial activity, but direct coating of silver onto a surface can be inactivated by biological anions with subsequent surface encrustation [14,15]. Previously reported studies have shown the benefit of silver impregnation in place of metallic silver deposition [16], although traditional techniques for the deposition or impregnation of silver employ high temperature processing, which limits

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substrate choice and increases costs [11,16]. In addition, one of the factors governing silver ion release from a coating matrix is the hydrophilicity of the coating [17]. Therefore, coatings with purely hydrophobic components will have limited water affinity and consequently a low silver release. This route may be ineffective, as coatings that release low levels of silver may select for resistance [18].

Polyether polyurethanes are low friction, biocompatible, elastomeric polymers that have been investigated as anti-fouling and biomedical coatings [19]. However, they are prone to biodegradation and environmental stress-cracking due to interactions with lysomal oxygen-free radicals and transition metal ions from implanted devices [20]. Polyether urethanes containing silver nanoparticles have shown improved biostability due to the free radical scavenging ability of the silver nanoparticles [21]. Modification of these polymers with fluorocarbons has been reported to improve biostability as fluorocarbons are stable against hydrolysis and oxidation [22].

In this work, we present the preparation, antibacterial activity and surface analysis of silver doped perfluoropolyether-urethane coatings. Silver ion release from these fluorinated dispersions exhibited significant antibacterial activity against MRSA, *E. coli*, *P. aeruginosa* and *Acinetobacter baumannii*. SEM was employed to examine the surfaces of the doped and undoped coatings after exposure to a biofilm-positive strain of *S. epidermidis.* XPS surface analysis provided information on the elemental and chemical composition of the coatings before and after exposure to different media. The surface composition of the silver doped fluoropolymers did not display significant changes in surface chemistry after six days bacterial exposure. In contrast, the blank fluoropolymers showed increased surface carbon and sodium levels, with a reduction in fluorinated content. The antibacterial capacity of the silver doped fluoropolymers was challenged by exchanging the nutrient rich medium every 24 h. After six days bacterial exposure, the supernatant broth still contained enough bio-active silver ions to inhibit planktonic *S. epidermidis*. In summary, these room temperature processed coatings may be employed in reducing contamination and biofouling on short-term indwelling devices and environmental surfaces.

2. Experimental

Silver doped fluoropolymers were prepared by the addition of a silver nitrate salt to an anionic perfluoropolyether (PFPE) urethane polymer, Fluorolink® P56 ($M_w \approx 20{,}000$, Solvay) [23]. 55 mg (0.3 mmol) silver nitrate (Aldrich) was dissolved by sonication in 2.5 ml (0.2 mol) de-ionised water containing 2.5 g polyaziridine cross-linker CX-100 (Neoresins). This solution was slowly added to the anionic fluorinated dispersion (95 g) under stirring. A silver nitrate free control was similarly prepared. The silver doped fluoropolymers were prepared in amber glassware to prevent photoreduction of the silver ions. The resulting silver doped and undoped polymers were applied to pre-cleaned tin coated steel and glass substrates using a 100 μm film applicator and cured at room temperature for 4 h, resulting in nominal film thickness of approximately 30 μm. The prepared coatings showed no tendency towards delamination or cracking before or after testing.

2.1. Instrumentation

The release of silver from the coatings was determined by GF-AAS using a Varian 110 Spectrometer equipped with a silver hollow cathode lamp. Microanalysis of the coatings was assessed using a Jeol 8600 scanning electron microscope (SEM). The samples were mounted on stubs and gold coated for SEM imaging at 10 KeV. XPS analysis employed an ESCALAB Mk II spectrometer equipped with a twin anode X-ray source was used to analyse the surface composition (≈10 nm) of the fluoropolymers and silver doped fluoropolymers. The instrument was equipped with a twin anode X-ray source (AlKα/MgKα) and an Alpha 110 analyser. In this work the twin anode AlKα X-ray source ($h\nu$ = 1486.6 eV) was used at 300 W (15 kV × 20 mA). Quantitative surface chemical analyses were calculated from the high resolution, core level spectra following the removal of a non-linear (Shirley) background. All spectrum binding energies were referenced to the hydrocarbon C 1s peak at 285 eV to correct for electrostatic charging effects during acquisition. Sample mounting for XPS analysis was achieved by fixing a specimen to a VG sample stub using double sided adhesive tape.

2.2. Silver release

Silver doped perfluoropolyether coated glass substrates (25 cm^2) were immersed in 50 ml of physiological buffered saline (PBS) at 37 °C initially for 1 h, followed by six successive 24 h immersion periods. Fresh media was used for each immersion period. Silver values are expressed in ppb (μg/L). The fluids were collected after each immersion period and analysed using GF-AAS.

2.3. Antibacterial activity

The antibacterial activity of the silver doped and undoped fluoropolymers, against a selection of Gram-positive and Gram-negative strains, was determined using a modified version of the Japanese standard (JIS Z 2801). These bacterial strains include MRSA (clinical isolate) *S. aureus* (ATCC 25922), *P. aeruginosa* (ATCC 27853), *A. baumannii* (clinical isolate) and a biofilm-positive strain of *S. epidermidis* (CSF 41498). Stock cultures of the bacteria were grown on plate count agar (PCA-3 w/v% tryptone, 1 w/v% glucose, 2.5 w/v% yeast, 9 w/v% agar, Oxoid). The organisms were grown overnight in nutrient rich broth to give a concentration of approximately 10^8 CFU/ml. These were diluted one in a hundred with maximum recovery diluent (MRD – 1 w/v% peptone, 8.5 w/v% NaCl, Oxoid) to give a working culture of approximately 10^6 CFU/ml. Doped and undoped coatings (25 cm^2) were inoculated with 400 μl of the working bacterial cultures and incubated at 37 °C overnight. The coatings were then agitated with MRD (20 ml) in sterile stomacher bags. To determine the number of colonies, the MRD was serially diluted tenfold and the resulting dilutions plated (100 μl) onto plate count agar (PCA) for overnight incubation at 37 °C.

2.4. Biofilm growth

Silver doped and undoped perfluorinated polymers were applied to pre-cleaned tin panels and allowed to cure for 4 h. Plastic wells were glued to the surface of the coatings using an epoxy fixative (Epofix) and left to cure for 24 h (Fig. 1). 5 ml of tryptic soy broth (TSB – tryptone 17 g/L, soy peptone 3 g/L, NaCl 5 g/L, dipotassium hydrogen phosphate 2.5 g/L, dextrose 2.5 g/L), supplemented with 0.25% glucose was added to the wells. 50 μl of approximately 10^8 CFU/ml *S. epidermidis* (CSF 41498) culture was added to the glucose modified TSB broth and the resulting bacterial broth poured into the wells and incubated for 24 h at 37 °C. The TSB medium was removed and replaced with fresh glucose modified TSB every 24 h and subsequently incubated at 37 °C. This process was repeated for six days. The daily exchange of nutrient rich media is useful in challenging the antibacterial capacity of the coating over the six days. Finally, the medium was decanted off and the coatings washed twice with sterile water. SEM was used to examine the surface of the coatings for the presence of any extraneous matter. Detailed surface elemental and chemical composition of the fluoropolymer coatings was obtained using XPS.