

Surface Modification

This disclosure relates to a novel composition for modifying the surfaces of, for example, medical devices such as catheters, stents, cannulas or other narrow tubing, to prevent bacterial infections and encrustations; medical devices comprising said surface; a method
5 for modifying said surfaces and methods of treatment that use said modified medical devices.

Background to the Invention

Medical devices are implanted into patients to treat a variety of diseases and conditions. Medical devices include catheters, stents [ureteral or prostatic stents], cannulas,
10 prosthesis and implants. The implantation of a medical device necessarily requires the exposure of the patient to both immune rejection of the implanted device and also an increased probability of an adventitious infection by a microbial pathogen. In addition, the accumulation of debris around and in the medical device can hinder the function of the device to an extent that the patient's health is placed at further risk. The interface
15 between the device surface and the surrounding environment is critical in providing a device that functions continuously and does not expose the patient to unnecessary risk of infection by microbial pathogens. In particular, the increase in antibiotic resistant bacterial pathogens such as *Staphylococcus aureus*, *Clostridium difficile*, *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Escherichia coli* (*E. coli*) has created a crisis in hospitals
20 where nosocomial infections are becoming prevalent. There is therefore a continued desire to provide medical implants that reduce opportunistic infection by microbial pathogens, in particular microbial pathogens that have become antibiotic resistant.

The longer a medical device stays in the body the higher the risk of infection will be for the patient. Nearly all patients undergoing long-term catheterization will develop
25 bacteriuria,¹ and catheter-associated urinary tract infection (CAUTI) is the most common healthcare-associated infection. Similarly, ureteral stents are found to be colonized by bacteria only after 2 weeks, and the rate increases with longer periods of stenting.² *E. coli* and *P. aeruginosa* are two of the most common pathogens found in both catheter- and ureteral stent-associated urinary tract infections.^{3,4}

30 Encrustation is another complication associated with urinary catheters and ureteral stents and is the major cause of mortality and morbidity in CAUTIs. If there is no obstruction in the catheters, CAUTIs are generally asymptomatic. However, if an obstruction occurs, the risk of ascending infection (i.e. pyelonephritis or kidney infection), bloodstream infection and even death will be increased.⁵ Pathogens like *Proteus mirabilis* (*P. mirabilis*) produce

urease, which hydrolyzes urea into ammonia, resulting in an increase in the pH of the urine. Under alkaline conditions, crystals of calcium and magnesium phosphates are formed and deposited on the device surface.⁶ Thus, the catheter or stent lumen will be obstructed by encrustation, which results in device malfunction.

5 In order to prevent bacterial infections and encrustation, antibacterial surface coatings of the catheter with diffusible antimicrobial agents have been used. Antimicrobial agents such as nitrofurazone⁷ and triclosan^{8,9} have been used in the manufacture of antibacterial urinary catheters and ureteral stents but the limited number of studies and the short-term results presented do not indicate long-term efficacy⁷⁻⁹ and moreover, the lack of a precise
10 dose management when using an antibiotic coated catheter can promote bacterial resistance. Silver has been widely used for antibacterial device coatings,¹⁰ however, commercially available silver coated urinary catheters have limited efficacy in inhibiting bacterial biofilm formation⁷ and encrustation.¹¹⁻¹³ One possible reason for their failure is the limited release of silver from the coating,¹² either due to low intrinsic silver release
15 capability¹² or the formation of a conditioning film (containing precipitated salts from urine and/or debris from lysed bacterial cells) on the catheter surface which blocks silver diffusion.¹³

Coating of medical devices is known in the art and is utilised to surface coat medical devices such as catheters with antibacterial materials and other bioactive agents.
20 US8308699 discloses substrates coated with one or more undercoats comprising e.g. methacrylate derivatives. The undercoat provides non-fouling and tethering components to immobilise onto the substrate and attach one or more top coats. A topcoat comprising zwitterionic materials such as glycine betaine or trimethylamine oxide can be used to improve biocompatibility and reduce fouling by proteins or bacteria. Bioactive agents such
25 as, fluorescent or colorimetric labels, antithrombotic labels or antimicrobial peptides can also be attached to the undercoat or topcoat. US20110305898 discloses biocompatible coatings for medical device substrates comprising non-fouling polymeric materials and particles of silver.

Dopamine, a small molecule compound, comprising catechol (DOPA) and amine (lysine)
30 groups has been found to have excellent adhesive properties and has since been used for many biomedical applications. Catechols and their derived compounds can self-assemble on various inorganic and organic materials, including noble metals, metal oxides, mica, silica, ceramics and even polymers.

This disclosure relates to a combination of a polymer for sustaining the release of antimicrobial agents, hereinafter referred to as a sustained release polymer, such as polydopamine (PDA), with an antimicrobial metal, for example silver, gold or copper as either ions or nanoparticles, in a bilayer, or multiple bilayers, on substrates such as silicone, is an effective means for efficiently preventing bacterial growth on the surface of medical devices such as catheters. The unique multi-layered modified surface provides sustained release of an effective antibacterial amount of silver, which when combined with an anti-fouling layer hinders effectively bacterial adhesion, biofilm formation, and encrustation in or on modified medical devices.

10 Statement of the Invention

According to an aspect of the invention there is provided a medical device comprising a support substrate having a modified surface, said surface comprising one or more layers covering all or part of the device surface, said layer[s] comprising a sustained release polymer associated with one or more antimicrobial metal ions or nanoparticles and a biocompatible uppermost layer comprising an anti-fouling polymer wherein, when in use, the modified surface inhibits bacterial adhesion and/or biofilm formation and encrustation of the medical device.

In a preferred embodiment of the invention the device surface comprises a first layer comprising said sustained release polymer and an antimicrobial metal ion[s] or nanoparticle[s] and a second layer, contacting said first layer, comprising a sustained release polymer wherein said second layer is provided with a biocompatible uppermost layer comprising an anti-fouling polymer to provide a modified medical device surface.

In a preferred embodiment of the invention said device surface comprises a plurality of first and second layers to provide a multi-layered device surface.

In a preferred embodiment of the invention the thickness of the modified surface is 2-20 μm . Preferably, the thickness of said modified surface is 10 μm +/- 10%.

In a preferred embodiment of the invention first and second layers comprise the same sustained release polymer.

In an alternative preferred embodiment of the invention the first and second layers comprise a different sustained release polymer.

In a preferred embodiment of the invention said sustained release polymer is polydopamine or its functionally equivalent derivatives.

In a preferred embodiment of the invention said ion or nanoparticle comprises an antimicrobial metal selected from the group consisting of: silver, gold or copper.

In a preferred embodiment of the invention said ion[s] or nanoparticle[s] comprise the antimicrobial metal silver.

- 5 In a preferred embodiment of the invention said antimicrobial metal comprises at least 12 μg metal ion[s]/ cm^2 .

In a further embodiment of the invention said antimicrobial metal comprises 12-25 μg metal ion[s]/ cm^2 .

- 10 In a preferred embodiment of the invention the concentration of said antimicrobial metal is 4-40 $\mu\text{g}/\text{cm}^2$ of the modified surface.

- 15 In a preferred embodiment of the invention said biocompatible layer comprises a polymer selected from the group consisting of: poly(sulfobetaine methacrylate-co-acrylamide) [poly(SBMA-co-AAm)], other zwitterionic polymers and derivatives, polyethylene glycol, polyacrylic acid, poly(2-hydroxyethyl methacrylate), agarose and alginate, and their derivatives.

In a preferred embodiment of the invention said biocompatible layer comprises poly(SBMA-co-AAm).

- 20 In a preferred embodiment of the invention said support substrate comprises a biocompatible polymer selected from the group consisting of: silicone, nylon, nitinol, polyurethane (PU), thermoplastic polymers, latex and polyethylene.

In a preferred embodiment of the invention said support substrate comprises silicone.

In a preferred embodiment of the invention said support substrate comprises a metal.

In a preferred embodiment of the invention said support substrate comprises a metal selected from the group: stainless steel, cobalt-chrome (Co-Cr), titanium and its alloys.

- 25 In a further preferred embodiment of the invention said modified surface comprises at least one antimicrobial agent wherein said agent is not an antimicrobial metal.

In a preferred embodiment of the invention said antimicrobial agent is an antibiotic.

- 30 Examples of classes of antibiotics effective in the control of bacterial pathogens include, by example only, penicillins, cephalosporins, rifamycins, sulphonamides, macrolides and tetracyclines.

In an alternative preferred embodiment of the invention said antimicrobial agent is an antimicrobial peptide.

Preferably, said antimicrobial peptides are dermicidins, cecropins or defensins.

In a preferred embodiment of the invention said medical device is a catheter.

5 In a preferred embodiment of the invention said medical device is a stent, for example a ureteral or prostatic stent.

In a preferred embodiment of the invention said medical device is a cannula.

According to a further aspect of the invention there is provided a method for the fabrication of a substrate surface of a device comprising the steps:

- 10 i) contacting all or part of the surface of the substrate with a fluid comprising at least one sustained release polymer to coat the substrate with a first polymeric layer;
- ii) contacting the first polymeric layer with a fluid comprising at least one antimicrobial metal to coat the first polymeric layer with said antimicrobial metal; optionally
- 15 iii) repeating step i) and ii) one or more times;
- iv) contacting the bi-layered or multi-layered coated substrate with a fluid comprising at least one sustained release polymer for controlling the antimicrobial metal release and for anchoring the subsequent anti-fouling layer; and
- 20 v) contacting the coated substrate with a fluid comprising at least one anti-fouling polymer.

In a preferred method of the invention the substrate is immersed in a liquid comprising said sustained release polymer which is subsequently immersed in a liquid comprising
25 said antimicrobial metal.

In an alternative method of the invention the substrate is contacted with a liquid spray comprising said sustained release polymer which is subsequently contacted with an aerosolized fluid comprising said antimicrobial metal.

In a preferred method of the invention said sustained release polymer is polydopamine.

30 In a preferred embodiment of the invention said antimicrobial metal is silver.

According to a further aspect of the invention there is provided a device comprising a substrate obtained or obtainable by the method according to the invention.

According to a further aspect of the invention there is provided a modified substrate according to the invention for use in the manufacture of a medical device.

- 5 In a preferred embodiment of the invention said device is selected from the group consisting of: a catheter, stent, ureteral or prostatic stent, cannula or prostheses.

According to a further aspect of the invention there is provided a surgical method to treat a subject in need of catheterisation comprising implanting a medical device according to the invention into the subject.

- 10 In a preferred method of the invention said medical device is implanted into the urethra of said subject.

In an alternative preferred method of the invention said medical device is implanted at the prostate gland of said subject.

- 15 In an alternative preferred method of the invention said medical device is implanted into the ureter of said subject.

According to a further aspect of the invention there is provided a kit comprising:

- 20 i) device comprising a substrate;
ii) a first solution comprising a sustained release polymer;
iii) a second solution comprising an antimicrobial metal; and
iv) a third solution comprising an anti-fouling polymer.

- 25 Throughout the description and claims of this specification, the words "comprise" and "contain" and variations of the words, for example "comprising" and "comprises", means "including but not limited to", and is not intended to (and does not) exclude other moieties, additives, components, integers or steps. "Consisting essentially" means having the essential integers but including integers which do not materially affect the function of the essential integers.

- 30 Throughout the description and claims of this specification, the singular encompasses the plural unless the context otherwise requires. In particular, where the indefinite article is used, the specification is to be understood as contemplating plurality as well as singularity, unless the context requires otherwise.

Features, integers, characteristics, compounds, chemical moieties or groups described in conjunction with a particular aspect, embodiment or example of the invention are to be understood to be applicable to any other aspect, embodiment or example described herein unless incompatible therewith.

5 **Main Figures**

Figure 1 Schematic diagram illustrating (A) synthesis of poly(SBMA-co-AAm), (B) steps for modifying silicone catheter surface, and (C) structural layers of P3 coated catheter;

10 Figure 2 X-ray photoelectron spectroscopy (XPS) wide scan spectra of pristine silicone surface, PDA-, PDA-silver nanoparticle (AgNP)-, P1, P2, and P3 coated silicone surfaces. Insets show the N 1s core-level spectrum of each surface;

Figure 3 Water contact angle of pristine, and PDA-, PDA-AgNP-, P1, P2, P3 coated silicone surfaces. * denotes significant difference ($P < 0.05$) compared with pristine silicone
15 surface;

Figure 4 Mechanical property of (i) Dover™ silver-coated catheter, (ii) unmodified all-silicone catheter, (iii) P2 and (iv) P3 coated silicone catheters;

20 Figure 5 (A) Silver content of the different catheter segments; and (B) silver content of P3 coated catheter segments obtained at the positions shown (X1, X2, X3). Diagram on top indicates the position of segment X1, X2, X3 (1 cm each) cut from a 6.5 cm catheter segment. Segments of 0.25 cm on both ends were discarded. Silver content was determined via hot acid digestion and inductively coupled plasma-mass spectrometry
25 (ICP-MS);

Figure 6 (A) Confocal laser scanning microscopy (CLSM) images (volume view) of *P. mirabilis* biofilm on the intraluminal surface of pristine, PDA-poly(SBMA-co-AAm)-, and P3 coated catheter segments after incubation in culture medium containing 10^5 cells/ml for
30 24 h. Scale bars represent 100 μm . (B) Viable bacterial cells on 1 cm^2 surface of catheter segments after incubation in phosphate buffered saline (PBS) containing 10^8 cells/ml for 4 h (bacterial adhesion assay) or in culture medium containing 10^5 cells/ml for 24 h (biofilm formation assay). * and # denote significant difference ($P < 0.05$) compared with pristine silicone catheter and Dover™ silver-coated catheter, respectively;

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Figure 7 Silver release from 1 cm catheter segments after incubation in 2 ml of (A) sterilized

artificial urine over 7 days at 37°C and (B) artificial urine medium with *P. mirabilis* (10^5 cells/ml) over 7 days at 37°C. (C) 1 cm P3 coated catheter segment after incubation in 2 ml artificial urine medium with *P. mirabilis* (10^5 cells/ml) over 48 days at 37°C. Release medium was changed every day and silver concentration in the release medium was determined using ICP-MS. Arrows indicate the average time point when encrustation occurred;

Figure 8 (A) Encrustation assay of 1 cm catheter segments challenged with freshly prepared artificial urine inoculated with 10^5 cells/ml *P. mirabilis* (2 ml). Samples i, ii, iii, iv, v represent control (bacterial suspension without catheter segment), Dover™ silver-coated catheter, and P1, P2, P3 coated catheters, respectively. Turbidity indicates crystal formation (Sample i on Day 1, and Samples i, ii on Day 7). (B) Scanning electron microscopy (SEM) image and (C) energy dispersive X-ray spectroscopy (EDX) elemental analysis of the material collected from the turbid control (Sample i) on Day 1 by centrifugation;

Figure 9 Mean encrustation-free period for the different catheters. * denotes significant difference ($P < 0.05$) compared with Dover™ silver-coated catheter; and

Figure 10 SEM images of (A-F) cross-section of intraluminal coating and (G-I) intraluminal surface; (A-C) before encrustation test, (D-E & G-H) after 7 days encrustation test, and (F & I) after 40 days encrustation test; (J-L) are EDX analyses of surfaces shown in (G-I), respectively. (A, D, G, J): Dover™ silver-coated catheter; (B, E, H, K): P2 coated catheter; and (C, F, I, L): P3 coated catheter. Scale bars represent 10 μ m.

Supplementary Figures

Figure S1 Device used in surface lubricity test (left: schematic illustration, right: photograph of experimental setup; insets: top-view of catheter segment in the device);

Figure S2 XPS Ag 3d core-level spectra of the intraluminal surfaces of Dover™, and P3 coated catheter segments (a) before and (b) after hot acid digestion;

Figure S3 Schematic illustration of (A) device used in catheter friction test; (B) catheter movement through the device in the friction test; (C) catheter deformation in the bending test;

5 Figure S4 XPS wide scan and N 1s core-level spectra of poly(SBMA-co-AAm);

Figure S5 Field emission scanning electron microscopy (FESEM) images and EDX silver maps of PDA- and PDA-AgNP-coated silicone surfaces. Arrows indicate particles of self-polymerized PDA. Scale bars in FESEM image and EDX map represent 1 μm ;

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Figure S6 Atomic force microscopy (AFM) 3D images of the extraluminal surfaces of pristine silicone catheter, P2 and P3 coated catheter, and Dover™ silver-coated silicone catheter;

15 Figure S7 Surface lubricity property of different catheter segments;

Figure S8 CLSM images of *P. mirabilis* biofilm on extraluminal surface of pristine and P3 catheters. Catheter segments were incubated in culture medium containing 10^5 cells/ml for 24 h. Scale bars represent 100 μm ;

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Figure S9 Viable *E. coli* UT189 on 1 cm^2 surface of catheter segments after incubation in artificial urine containing 10^8 cells/ml for 4 h. * denotes significant difference ($P < 0.05$) compared with pristine silicone catheter;

25 Figure S10 Viable *E. coli* DH5 α and *P. mirabilis* on 1 cm^2 pristine and P3 coated PU and Co-Cr surfaces after incubation in culture medium containing 10^5 cells/ml for 24 h. * denotes significant difference ($P < 0.05$) compared with pristine substrate surface;

30 Figure S11 (A) Size distribution of particles in artificial urine after incubation with catheter segments for 24 h, and (B) silver concentration in artificial urine after incubation with catheter segments for 24 h before and after filtration, and bactericidal efficiency of the silver-loaded artificial urine against *P. mirabilis*;

35 Figure S12 SEM images of cross-section of intraluminal coating of (A) Dover™ silver-coated catheter after 7 days immersion in sterilized artificial urine, (B) P2 coated catheter after 7 days immersion in sterilized artificial urine, and (C) P3 coated catheter after 40 days immersion in sterilized artificial urine. Scale bars represent 10 μm ;

Figure S13 (A) XPS wide scan and Ag 3d spectra of extraluminal catheter surfaces before and after the stability tests. (B) CLSM images of *P. mirabilis* biofilm on extraluminal catheter surfaces after the stability tests. Scale bars represent 100 μm . P3-F and P3-B represent P3 catheter segments after the friction test and bending test, respectively; and

Figure S14 (A-B) Viability of 3T3 fibroblast cells after incubation in medium containing catheter extract. SA/Vol ratio is the ratio of catheter surface area to volume of medium used in extraction. (C) Silver concentration in the extraction medium as determined by ICP-MS. (D) Viability of 3T3 fibroblast cells after incubation in medium with different concentrations of AgNO_3 for 24 h and 72 h. Viability is expressed as a percentage relative to the result obtained with the non-toxic control. * denotes significant difference ($P < 0.05$) compared with non-toxic control.

Materials and Methods

All-silicone Foley catheters (Bardex[®], 14 Ch/Fr (OD 4.7 mm)) were purchased from C. R. Bard Inc., Georgia, US, and used for the surface modification experiment. Silver-coated
 5 100% silicone Foley catheters (Dover[™], 14 Ch/Fr (OD 4.7 mm)) were obtained from Covidien LLC, Massachusetts, US. According to the manufacturer, the Foley catheter is coated with phosphate ionic silver hydrogel on both intraluminal and extraluminal surfaces. Medical grade silicone sheets (1 mm thickness) were obtained from Bioplexus Inc., California, US. Polyurethane sheets (PU, 2 mm thickness) were purchased from
 10 Central Polymer Engineering Supply, Singapore. Cobalt-chrome alloy foils (Co50/Cr20/W15/Ni10/Fe3/Mn2, Co-Cr, 0.6 mm thickness) were purchased from Goodfellow Inc., Huntingdon, UK. Dopamine hydrochloride, silver nitrate, [2-(methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl) ammonium hydroxide (sulfobetaine methacrylate, SBMA), acrylamide (AAM), ammonium persulfate, 3-[4,5-dimethyl-thiazol-
 15 2-yl]-2,5-diphenyltetrazolium bromide (MTT), tryptic soy broth, lysogeny broth, nutrient broth and agar were purchased from Sigma-Aldrich, Missouri, US. All other chemicals used were analytical reagents (AR) and purchased from Sigma-Aldrich or Merck Chem. Co. (Darmstadt, Germany). *E. coli* (ATCC DH5 α), *P. mirabilis* (ATCC 51286, a strain isolated from a patient with urinary catheter infection) and 3T3 fibroblasts were obtained
 20 from American Type Culture Collection (ATCC, Virginia, US). *P. aeruginosa* PAO1 was purchased from National Collection of Industrial Food and Marine Bacteria (NCIMB, Aberdeen, UK). *E. coli* UT189, an uropathogenic strain isolated from a patient with uncomplicated cystitis¹⁴ was kindly provided by Dr. Swaine Chen of Genome Institute of Singapore.

25 Artificial urine basal solution was prepared according to the method reported in the literature.¹¹ Calcium chloride (0.49 g), magnesium chloride hexahydrate (0.65 g), sodium chloride (4.6 g), di-sodium sulfate (2.3 g), tri-sodium citrate dehydrate (0.65 g), di-sodium oxalate (0.02 g), potassium dihydrogen phosphate (2.8 g), potassium chloride (1.6 g),
 30 ammonium chloride (1.0 g), and urea (25 g) were dissolved in 800 ml distilled water. The pH was adjusted to 6.0 using 1 M sodium hydroxide solution and the solution was sterilized by filtration through a 0.2 μ m membrane. To prepare the artificial urine salt solution for testing the release of silver from the coated catheters, pre-autoclaved distilled water was added to the basal solution to bring the final solution volume to 1,000 ml. For
 35 artificial urine medium used in the encrustation test, 200 ml solution with 1.0 g tryptic soy broth and 5.0 g gelatin was prepared separately and sterilized by autoclaving, and then

added to the sterilized artificial urine basal solution to make up a total volume of 1,000 ml. The prepared solutions were kept in a 4°C refrigerator and used within one month.

Synthesis of poly(SBMA-co-AAm)

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Poly(SBMA-co-AAm) was synthesized via free radical polymerization according to a previously reported method (Figure 1A).¹⁵ Briefly, SBMA (3.35 g, 12 mmol), AAm (0.21 g, 3 mmol) and distilled water (20 ml) were placed in a 50 ml single-neck round-bottom flask. The mixture was constantly stirred at 500 rpm and degassed by purging with nitrogen for 20 min. Ammonium persulfate (23 mg, 0.1 mmol) was added to initiate the reaction, and degassing was maintained for another 10 min. The flask was then sealed, and the reaction was allowed to proceed in a 60°C oil bath for 5 h with continuous stirring. The product was subjected to dialysis using a cellulose membrane (molecular weight cut-off of 12,000, Sigma-Aldrich) for three days to remove the unreacted reagents, salts, and low molecular weight products, and then lyophilized.

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Surface modification

Silicone urinary catheters were cut into 6.5 cm length before the modification. The surfaces of the catheter segments were modified as illustrated in Figure 1B. A PDA layer was first coated on the catheter surface by immersing the catheter segment in 10 ml of dopamine solution (2 mg/ml in 10 mM Tris buffer, pH 8.5) at room temperature with shaking for 24 h.¹⁶ AgNPs were subsequently formed on the surface by immersing the PDA-coated segment in 10 ml of 50 mM AgNO₃ aqueous solution at room temperature with shaking for 24 h. Another PDA layer was then grafted by treating the PDA-AgNP-coated segment with 10 ml freshly prepared dopamine solution (2 mg/ml in 10 mM Tris buffer, pH 8.5) for 24 h. This modified catheter with PDA-AgNP-PDA layers was denoted as P1 coated catheter. The P1 coated catheter was further immersed in 10 ml poly(SBMA-co-AAm) solution (10 mg/ml in 10 mM Tris buffer, pH 8.5) in a 37°C water bath with shaking for 24 h, and denoted as P2 coated catheter (PDA-AgNP-PDA-(pSBMA-co-AAm)). A PDA-poly(SBMA-co-AAm)-coated catheter was also prepared by immersing the PDA-coated segment in poly(SBMA-co-AAm) solution in the same manner. The P3 coated catheter (PDA-(AgNP-PDA)₂-(pSBMA-co-AAm), Figure 1C) was prepared by treating the P1 coated catheter with AgNO₃ solution, dopamine solution, and poly(SBMA-co-AAm) solution sequentially for 24 h in each step as described above. After each treatment step, the coated catheter segments were washed with distilled water, and after completion of the coating process, the segments were dried under nitrogen flow and

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stored in the dark until further use. After modification of the 6.5 cm catheter segment, 0.25 cm segments from both ends of the catheter were cut off and the remaining part was cut into specific lengths according to the requirements of the different experiments (6 cm for testing of mechanical property and coating stability, 1 cm for the silver release and encrustation tests, and 1.5 cm for the bacterial adhesion and biofilm formation tests). Thus, only the intraluminal and extraluminal surfaces of these catheter segments were coated. The Dover™ silver-coated catheter was cut into similar segments and used for comparative purposes.

Surface modification of silicone and PU sheets (6.5×1 cm²) and Co-Cr foil (1×1 cm²) was also carried out. The Co-Cr foil was ultrasonically cleaned in dichloromethane, acetone and water for 10 min in each step before use. The coating procedures were as described above for the catheter segment, using 10 ml of reagent in each step for the silicone and PU sheets, and 2 ml for the Co-Cr foil.

Surface characterization

Due to the curved surface of the catheter, characterization of its surface with techniques such as FESEM, XPS analysis and contact angle measurement could not be readily carried out. Thus, most of these measurements were carried out with flat silicone sheets which were coated in the same manner as the silicone catheter. The surface compositions were analyzed using XPS on an AXIS Ultra^{DLD} spectrometer (Kratos Analytical Ltd, Manchester, UK) equipped with a monochromatized Al K α X-ray source (1468.6 eV photons). Contact angles of the different surfaces were measured at room temperature by the sessile drop method using a telescopic goniometer (Rame-Hart, New Jersey, US). A 3 μ l water droplet was applied on the surface, and the static contact angle was recorded. For each type of coating, at least nine samples were tested and the mean value and standard deviation (SD) of the contact angles were reported. The surface morphology of PDA- and PDA-AgNP-coated silicone sheets were observed using FESEM (JEOL, Model JSM-6700, Tokyo, Japan). The presence of silver on these surfaces was verified with XPS and EDX (JEOL, Model 5600LV scanning electron microscope with EDX detector from Oxford Instrument, Oxford, UK). The surface topography of the extraluminal surface of the different catheter segments was characterized in the dry state by AFM (Nanoscope IIIa AFM, Digital Instruments, Inc., New York, US). The root-mean-square (RMS) roughness of the surfaces was calculated from the roughness profile determined by AFM.

Mechanical property and surface lubricity tests

Tensile testing of the catheter segments was performed using an Instron universal materials testing machine (Model 5544, Massachusetts, US). The catheters were cut into lengths of 6 cm, and each segment was clamped at 2.5 cm from each end such that a 1 cm segment remained between the clamps. The segments were pulled at room temperature at 10 mm/min until break point. The stress (force per cross-sectional area) versus strain curve was recorded for each catheter segment.

The surface lubricity assay was carried out in a similar manner as an earlier report¹⁷ with slight modification using the device shown in Figure S1. The catheters were cut into segments of 4 cm and equilibrated in distilled water for 10 min prior to the test. One end of the catheter segment (1 cm length) was clamped vertically in the Instron universal materials testing machine. The remaining portion of the catheter segment (3 cm) was sandwiched between two pieces of silicone sheets (thickness: 1 mm, L×W: 5.5 cm×3 cm) which were pre-fixed onto two pieces of polymethyl methacrylate (PMMA) plates (L×W: 5.5 cm×3 cm) at the position just below the Instron clamp. The two PMMA plates formed a channel of 6 mm diameter. Since the thickness of each silicone sheet was 1 mm, the diameter of the channel for the catheter would be ~4 mm. Therefore, the surface of catheter segment (catheter diameter: 4.7 mm) fully contacted the silicone sheets in the channel. The PMMA plates, silicone sheets and catheter segment were tightly fixed using a metal screw clamp with a ballasting weight in the bottom. Throughout the experiment, a stream of distilled water (0.5 ml/min) was directed at the catheter segment and the channel at the top of the PMMA plates to maintain a wet environment. The segment was pulled at 5 mm/s, and the force versus distance moved was recorded for each segment. The work required to pull out the segment from between the fixed silicone sheets was calculated from the integrated area under each curve using Origin 8.1 software. Three replicate measurements were performed for each type of catheter.

Bacterial adhesion and biofilm formation assay

Bacteria were cultured in broth overnight (lysogeny broth for *E. coli* UT189 and *P. aeruginosa*, tryptic soy broth for *P. mirabilis*, nutrient broth for *E. coli* DH5α) and then harvested by centrifugation (2,700 rpm, 10 min) followed by washing twice with PBS (10 mM, pH 7.4). The bacterial cells were resuspended in PBS (10 mM, pH 7.4) at a concentration of 10^8 cells/ml, corresponding to an optical density of 0.1 at 540 nm as determined in our earlier work.¹⁶ This bacterial suspension was directly used for the

bacterial adhesion assay. For the biofilm formation assay, the harvested bacterial cells were resuspended in the appropriate broth at a concentration of 10^5 cells/ml. A 1.5 cm catheter segment (pristine and modified) was placed in a 15 ml centrifuge tube with 2 ml of the bacterial suspension and incubated in a 37°C water bath with shaking at 100 rpm.

5 The duration of incubation was 4 h for the bacterial adhesion assay, and 24 h for the biofilm formation assay. After the incubation period, the bacterial suspension was removed and the segment was washed three times with PBS to remove any non-adhered or loosely adhered bacteria. For the spread plate method, to discount the bacteria adhering on the uncoated cross-sectional surface at the ends of catheter segment, the
10 two ends (0.25 cm each) of the catheter segment were sliced off. The remaining 1 cm segment was put in a new 15 ml centrifuge tube with 3 ml PBS. The PBS solution with the segment was subjected to ultrasonication for 7 min and vortex for 20 s to dislodge the adherent bacterial cells. The bacterial solution was serially diluted, spread on the appropriate agar plate (lysogeny agar for *E. coli* UTI89 and *P. aeruginosa*, tryptic soy
15 agar for *P. mirabilis*, nutrient agar for *E. coli* DH5 α) and cultured overnight to determine the number of bacterial cells. Bacterial adhesion on the catheter surface in artificial urine was performed in a same manner with sterilized artificial urine instead of PBS used for preparation of the bacterial suspension. All experiments were performed in duplicate with three samples and the mean values were calculated.

20 The biofilm formation assay was also carried out with pristine and P3 coated PU sheet and Co-Cr foil. The general procedure is similar to that described above for the catheter segment. PU sheet or Co-Cr foil of 1×1 cm² was incubated in 2 ml of *E. coli* DH5 α or *P. mirabilis* suspension (10^5 cells/ml in the appropriate broth mentioned above) for 24 h, and
25 the number of bacterial cells on the substrate surface was then determined using the spread plate method.

The biofilm on the intraluminal and extraluminal surfaces of the catheters was also observed using CLSM. The catheter segments after biofilm assay were washed with PBS
30 three times and cut lengthwise and fixed on a glass slide using double-sided tape to expose the selected surface (intraluminal or extraluminal). A combination dye (LIVE/DEAD BacLight bacterial viability kits, Molecular Probes, L13152, Life Technologies, California, US) was then applied according to the manufacturer's instruction. The live bacteria with intact membranes in the biofilm would be stained green, which was then
35 observed under a Nikon Ti-E microscope with A1 confocal system (Nikon, Tokyo, Japan). A Multi-Argon 488 laser was used as the source of illumination, with 488 nm excitation

and long-pass 500-530 nm emission filter settings for green signal. NIS-Elements C software was used to generate the volume view images of the biofilms.

Determination of silver content and release profile in sterilized artificial urine

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Silver in the catheter coating was extracted using a slightly modified acid digestion method and the content was determined using ICP-MS (Model HP 7500a, Agilent, California, US).¹⁸ Briefly, a 1 cm catheter segment was immersed in 10 ml of 50% HNO₃ in a glass bottle with a watch glass cover. The silver coating on both the intraluminal and extraluminal surfaces of the catheter segment was digested by heating the acid solution to 100°C in an oil bath, and the temperature was maintained for 1 h to complete the digestion process. The original colour of the silver-coated catheters (orange for DoverTM silver-coated catheter and dark brown for P1, P2, and P3 coated catheters) faded after digestion and the segment became fragile. The intraluminal surface of the digested catheter segment was tested using XPS to check for complete dissolution of silver from the coating (Figure S2). The digested solution was diluted with distilled water, and silver was quantified using ICP-MS based on a reference calibration curve generated from solutions of different silver concentrations prepared from a standard silver solution (1,000 mg/l Ag in nitric acid, Sigma-Aldrich). Three replicates were measured, and the results were expressed as mean ± SD.

Silver release was carried out by immersing a 1 cm catheter segment in 2 ml of sterilized artificial urine in a 15 ml centrifuge tube. The solution was incubated in a 37°C water bath with shaking at 100 rpm. Every 24 h, the catheter segments were taken out and immersed in a fresh artificial urine solution. The solution containing the released silver was collected and stored in a 4°C refrigerator. 1 ml of 2% HNO₃ was added to the collected solution before ICP-MS measurement. The total amount of silver released over 7 days was determined by summing up the amount released each day. Three replicates were measured, and the results were expressed as mean ± SD.

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Determination of silver species in artificial urine and their bactericidal properties

A 1 cm catheter segment was immersed in 2 ml of sterilized artificial urine in a 15 ml centrifuge tube, which was then incubated in a 37°C water bath with shaking at 100 rpm. After 24 h, the catheter segments were taken out and the artificial urine containing the released silver was collected. The size distribution of any particles in the artificial urine was recorded using a Zetasizer nanosystem (Malvern Instrument, Worcestershire, UK).

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The artificial urine containing the released silver was also filtered through a 0.2 μm membrane, and ICP-MS was used to determine the silver concentration in the filtrate. To evaluate the bactericidal efficiency of the silver species, 0.9 ml of the artificial urine with released silver (before and after filtration through the 0.2 μm membrane) was mixed with 0.1 ml of *P. mirabilis* suspension (5×10^7 cells/ml in artificial urine) in a 15 ml centrifuge tube. After 4 h shaking at 100 rpm in a 37°C water bath, the bacterial suspension was spread on tryptic soy agar plate and cultured overnight to determine the number of viable bacteria. Control experiment was carried out by mixing 0.9 ml of sterilized artificial urine with 0.1 ml of *P. mirabilis* suspension (5×10^7 cells/ml in artificial urine). The bactericidal efficiency of the released silver species was calculated from the reduction in the number of viable bacterial cells in the artificial urine with these species as compared to that in the control experiment.

Encrustation assay

A 1 cm catheter segment was immersed into 2 ml artificial urine medium with *P. mirabilis* at a concentration of 10^5 cells/ml. The solution was incubated in a 37°C water bath with shaking at 100 rpm. After every 24 h, the segment was taken out and immersed in another 2 ml of freshly prepared artificial urine medium with 10^5 cells/ml *P. mirabilis*. The bacterial challenge continued until the medium was visually observed to turn turbid as a result of precipitate formation. This stage was considered as the onset of encrustation. For each type of coating, five independently prepared samples were tested and the time taken for encrustation to occur was recorded. The artificial urine medium used in the test was collected each day and stored in a 4°C refrigerator. 1 ml of 10% HNO_3 was added to each tube and the silver concentration in the solution was determined using ICP-MS as described above. Three replicates were measured, and the results were expressed as mean $\pm$ SD. The cross-section and intraluminal surface of the catheter segment before and after the encrustation test were observed using SEM (JEOL, Model 5600LV, Tokyo, Japan). The coating thickness was measured from three images of each type of coating using the Nano Measurer software, and six different positions in each image were measured. The elements present on the intraluminal surface of the catheter segment after the encrustation test were determined using EDX.

Stability test

Friction and bending tests were carried out to assess the stability of the surface coating of the P3 coated catheter. The device used in the friction test is shown schematically in

Figure S3A. Fresh pig bladder was slit open to gain access to the inside cavity, and sandwiched between two pieces of PMMA plate (L×W: 5.5 cm×3 cm). The two PMMA plates formed a channel of 6 mm diameter for the catheter to traverse through the device. The PMMA plates and pig bladder were tightly fixed using two cable ties. A 6 cm catheter segment was inserted into the bladder cavity from one side, and then pulled out from the other side (Figure S3B). The procedure was repeated five times to achieve a total travelling distance of 30 cm, which is approximately equivalent to the traversing distance of the catheter in the urinary tract during catheterization. The bending test was carried out by repeated bending a 6 cm catheter segment into a circle upward and downward fifteen times (Figure S3C). A 2 cm segment was cut from the middle part of the 6 cm catheter segment after the bending test for the subsequent investigations. The catheter segments after the tests were washed with copious amounts of distilled water and dried under nitrogen flow. XPS analysis was used to compare the surface composition of P3 coated catheter before and after the friction test (P3-F) and bending test (P3-B). To further evaluate the antibacterial efficacy of catheter after the stability tests, the catheter was cut into 1.5 cm segments and subjected to biofilm formation assay. After 24 h, the catheter was cut lengthwise and fixed on a glass slide using double-sided tape with the extraluminal surface facing upwards. The biofilm formed on the extraluminal surface was stained using with LIVE/DEAD BacLight dye and observed using CLSM (see above for details of XPS characterization and biofilm formation assay).

Cytotoxicity assay

The cytotoxic effect of the modified catheters on mammalian cells was evaluated using the MTT assay in accordance with the standard protocol indicated in ISO 10993-5 (Biological evaluation of medical devices-Part 5: Tests for *in vitro* cytotoxicity).¹⁹ Catheter segments to be tested were first sterilized under UV irradiation for 1 h. To extract any potentially toxic substance, the catheter segments were placed in a 15 ml tube containing the culture medium for 3T3 fibroblasts (described below). Different catheter surface area/volume of medium (SA/Vol) ratios were used (with one 1 cm catheter segment in 2 ml culture medium, SA/Vol ratio = ~1.25 cm²/ml; with two 1 cm catheter segments in 1.7 ml culture medium, SA/Vol ratio = ~3 cm²/ml). The catheter segments were incubated at 37°C in a humidified atmosphere of 5% CO₂ and 95% air for 72 h. The concentration of extracted Ag in the medium was determined using ICP-MS as described above.

3T3 fibroblast cells were cultured in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 1 mM L-glutamine, 100 IU/ml penicillin. 100

5 μ l of the cell suspension was seeded in a 96-well plate at a density of 10^4 cells per well and incubated at 37°C in a humidified atmosphere of 5% CO₂ and 95% air for 24 h. 100 μ l of the catheter extract medium was then used to replace the medium in each well and the cells were incubated at 37°C for either another 24 h or 72 h. Control experiments were carried out for similar incubation times using only culture medium (the medium was also pre-incubated under the same condition but without any catheter segment) as the non-toxic control, and medium with 1% Triton X-100 (Sigma-Aldrich) as the toxic control. At the end of the incubation period, the culture medium in each well was removed, and 100 μ l of MTT solution (0.5 mg/ml in medium) were added to each well. After 4 h of incubation at 37°C, the medium was removed and the formazan crystals were dissolved in 100 μ l of dimethyl sulfoxide for 15 min. The optical absorbance was then measured at 570 nm on a microplate reader (Tecan GENios, Männedorf, Switzerland). The results were expressed as percentages relative to the optical absorbance obtained in the non-toxic control experiments. To determine the cytotoxicity of silver, AgNO₃ was dissolved in the culture medium at different concentrations. This Ag-containing medium was then used to culture the cells which were pre-seeded in the 96-well plate, and the cell viability was determined using MTT assay as described above.

Statistical analysis

20 The results were reported as mean $\pm$ SD and were assessed statistically using one-way analysis of variance (ANOVA) with Tukey post hoc test. Statistical significance was accepted at $P < 0.05$.

25 Example 1

Composition of poly(SBMA-co-AAm)

Figure S4 shows the XPS wide scan and N 1s core-level spectra of poly(SBMA-co-AAm). With a monomer feed ratio of [SBMA]/[AAm] at 4:1, the [N⁺]/[N] ratio of poly(SBMA-co-AAm) was determined to be 3.2 from XPS analysis. As there is one -N⁺(CH₃)₃ group in each SBMA unit, and one -NH₂ group in each AAm unit, the ratio of [SBMA]/[AAm] in poly(SBMA-co-AAm) was deemed to be 3.2. The lower SBMA content in the co-polymer compared to the monomer feed ratio was attributed to the decrease in reactivity of the SBMA monomer arising from steric hindrance which restricted the access of the larger SBMA monomer to the growing polymer chain as compared to the smaller AAm monomer.²⁰

Example 2

Coating process

Our previous work has shown that dopamine can self-polymerize to PDA and form a highly stable layer on silicone surface for anchoring other moieties.¹⁶ Immersion of a PDA-coated surface in an AgNO₃ aqueous solution reduced the Ag⁺ ions into nanoparticles. FESEM images and EDX analysis confirmed that the AgNPs were distributed uniformly at high density on this surface (Figure S5). A second PDA layer can then be coated over the AgNPs via grafting with the underlying PDA layer and/or binding with the AgNPs.^{21,22} With the flexibility offered by PDA, additional AgNP-PDA bilayers can thus be formed on top of the PDA-AgNP-PDA surface. Poly(SBMA-co-AAm) (ratio of [SBMA]/[AAm] in poly(SBMA-co-AAm) was determined to be 3.2, Figure S4), an antifouling polymer, can also be grafted via Michael addition and Schiff-base reactions between the amino groups in poly(SBMA-co-AAm) and the catechol/*o*-quinone groups in the PDA layer.^{16,21}

Example 3

XPS analysis of pristine and modified silicone sheets

Figure 2 shows the XPS wide scan and N 1s core-level signals of pristine and modified silicone sheet surfaces after the various coating steps. As can be seen, there was no detectable nitrogen signal on pristine silicone surface. After 24 h of treatment with PDA, a strong N 1s peak at ~400 eV was observed, indicating the grafting of a PDA layer. After formation of AgNPs on the PDA coating, the Ag 3d peaks at ~374.1 eV and ~368.1 eV could be observed. The decrease of the N 1s peak at ~400 eV of PDA-AgNP was attributed to the coverage of the PDA by the AgNPs. When another PDA layer was coated on the PDA-AgNP coating, the N 1s signal increased and Ag 3d signal decreased correspondingly. On the P2 and P3 coated surfaces, the presence of the poly(SBMA-co-AAm) graft layer was indicated by the S 2p and S 2s peaks (~167.5 eV and ~231.5 eV) in the wide scan, as well as the additional N 1s peak at ~402.5 eV, correspondingly to the –N⁺(CH₃)₃ groups (Figure 2).

Example 4

Contact angle of pristine and modified silicone sheets

The contact angles of the pristine and modified silicone sheet surfaces differed greatly (Figure 3). When the hydrophobic silicone surface was coated with PDA, its hydrophilicity

increased very significant (contact angle decreased from $106.1 \pm 1.6^\circ$ for pristine surface to $52.7 \pm 2.6^\circ$ for PDA-coated surface), and with AgNPs deposition, the contact angle showed a further slight decrease to $46.0 \pm 3.7^\circ$. An additional coating of PDA on the AgNP layer (P1) reduced the contact angle to $30.2 \pm 3.8^\circ$. After coating with poly(SBMA-co-AAm) layers (P2 & P3), the surfaces were fully hydrated in water (contact angle $< 10^\circ$), as a result of the large number of zwitterionic groups on the surface.^{23,24}

Example 5

Surface roughness of pristine and modified silicone catheters

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The surface roughness of catheter shows significant changes after the various modification steps (Figure S6). The pristine silicone catheter surface is relatively smooth with the roughness of 12.5 nm. With the P2 and P3 coatings, the catheter surface became rougher, and the roughness value increased to 147 nm and 179 nm, respectively. In comparison, the phosphate silver ionic hydrogel coating on the Dover™ catheter surface has a surface roughness of 112 nm.

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Example 6

Mechanical properties and surface lubricity

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Indwelling devices such as urinary catheters and ureteral stents have to withstand body movements during their placement *in vivo*, and thus, the catheters have to retain their mechanical integrity after surface modification. Figure 4 shows a comparison of the mechanical property of the Dover™ silver-coated silicone catheter, the unmodified all-silicone catheter, and the P2 and P3 coated silicone catheters. The stress-strain relationship for the P2 and P3 coated catheters was not much different from that of the unmodified all-silicone catheter. Thus, our coating process did not significantly alter the mechanical property of the silicone. On the other hand, the Dover™ silver-coated silicone catheter required a higher stress to produce a similar strain as the all-silicone and P2, P3 catheters, and it also reached the break point after a lower strain.

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A comparison of the surface lubricity of the Dover™, pristine, P2 and P3 catheter segments is shown in Figure S7. For the pristine catheter segment, the work required to pull the catheter segment out from the fixed silicone sheets was 1033.5 ± 57.9 N·mm (integrated area below the curve). For the P2 and P3 coatings, 485.5 ± 26.3 N·mm and 481.3 ± 35.3 N·mm were required, respectively. Since the catheter segments are made from silicone, which is an elastomer, the segment may elongate when it is pulled between

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the fixed silicone sheets. This can be seen in Figure S7 where the distance traversed was greater than the length of the initial catheter segment. Nevertheless, since the mechanical properties of the pristine, P2 and P3 catheters were comparable (Figure 4), the differences between the lubricity curves were mainly due to the external frictional forces.

5 These results indicated the coated catheter surface have become more lubricious than that of the pristine catheter. In the presence of water, the PDA coating is reported to be hydrated and has low frictional force.²⁵ Similarly, the poly(SBMA-co-AAm) layer will also be highly hydrated. Hence, it may be expected that in the *in vivo* environment, the P2 and P3 coatings will impart lubricity to the catheter surface, which would be advantageous during catheter insertion and removal. For the Dover™ catheter segment, 277.6±22.7 N·mm was required to move it out from the fixed silicone sheets. Since the Dover™ catheter was more rigid than the unmodified, P2 or P3 silicone catheters as shown in Figure 4, it underwent a smaller degree of deformation and a shorter distance was traversed during the lubricity test. Thus, comparison of the work required for the Dover™
10 catheter and the other catheters shown in Figure S7 may not give an accurate indication of differences in surface lubricity.
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Example 7

Quantification of silver content in catheter coating

20 Figure 5A shows the silver content in the different catheter segments. The Dover™ silver-coated catheter has a silver content of 10.2±0.6 µg/cm², while the P1 and P2 coated catheters have slightly higher amounts (13.2±0.5 and 12.8±0.8 µg/cm², respectively) and the P3 coated catheter has a much higher silver content of 21.9±0.7 µg/cm². Figure 5B
25 shows there was no significant difference in silver content among 1 cm segments cut from different positions of a 6.5 cm P3 coated catheter segment, which confirmed the uniformity of the coating over the catheter segment.

Example 8

Bacterial adhesion and biofilm formation assay

30 Bacteria adhered and formed biofilm readily on the pristine catheter as shown by the CLSM image (volume view) of the *P. mirabilis* biofilm on the intraluminal surface of the pristine catheter in Figure 6A. The PDA-poly(SBMA-co-AAm) coating reduced biofilm
35 formation but a layer of bacterial cells could still be observed on the surface. With the presence of silver in the coating (P3 coated catheters), biofilm formation was further inhibited and much fewer live bacteria existed on the surface. A similar reduction in

biofilm formation on the extraluminal surface of the P3 coated catheter as compared to that on the corresponding surface of the pristine catheter was also observed (Figure S8).

The efficiency of the different types of modified catheter surfaces in inhibiting bacterial colonization is given in Figure 6B. As can be seen, bacterial adhesion and biofilm formation on the PDA-coated catheter surface was similar to that on the pristine silicone catheter. On the other hand, the PDA-poly(SBMA-co-AAm)-coated catheter reduced bacterial adhesion and biofilm formation by > 96% for *E. coli* UT189, > 97% for *E. coli* DH5 α , > 92% for *P. mirabilis*, and > 94% for *P. aeruginosa* compared to that on the pristine silicone catheter. These results are consistent with earlier findings that zwitterionic polySBMA coating can effectively reduce bacterial adhesion and biofilm formation.²⁶ With a combination of the anti-adhesive property of the poly(SBMA-co-AAm) coating and the bactericidal nature of the released silver species, the adhesion of the four bacterial strains on the P2 and P3 coated catheters was reduced by $\geq$ 99%. The extent of *E. coli* UT189, *E. coli* DH5 α and *P. aeruginosa* biofilm formation was also reduced by $\geq$ 99%. Although *P. mirabilis* has been reported to have a higher propensity to form biofilm than *E. coli*,^{3,16} the P2 and P3 coated catheters could still achieve $\geq$ 98% reduction in *P. mirabilis* biofilm formation. The commercially available DoverTM silver-coated catheter reduced bacterial adhesion by $\geq$ 99% and biofilm formation by $\geq$ 97% for the four bacterial strains. The bacterial adhesion results shown in Figure 6B were obtained using bacterial suspensions in PBS. A similar assay was carried out using *E. coli* UT189 suspension in artificial urine instead of PBS. The results obtained were similar to those obtained with PBS, and the DoverTM and P3 coated catheters reduced *E. coli* UT189 adhesion by > 99% compared to the pristine silicone catheter (Figure S9). Furthermore, the strategy of using multiple AgNP-PDA bilayers coupled with a final grafted layer of poly(SBMA-co-AAm) can be adapted for coating other materials such as PU and Co-Cr, and the biofilm formation by *E. coli* DH5 α and *P. mirabilis* on the coated surfaces reduced by at least 97% compared to the respective unmodified surface (Figure S10).

Example 9

Silver release profile in sterilized artificial urine

The silver release profiles from the coated catheter segments in sterilized artificial urine are shown in Figure 7A. The release profile of silver from the P1, P2, and P3 coated catheter segments was fairly linear over 7 days. The P1 and P2 coated catheter segments have similar silver content (13.2 ± 0.5 and 12.8 ± 0.8 $\mu\text{g}/\text{cm}^2$, respectively, Figure 5), and the amounts of silver released in the first two days were comparable, but the latter

maintained a more constant release rate after that. The antifouling property of the poly(SBMA-co-AAm) layer in the P2 coated catheter segment may inhibit the deposition of precipitated salts from urine on the surface and thus, allowing the diffusion of Ag^+ to occur more freely. Although the silver content in the P3 coating is much higher than those in P1 and P2 coatings ($21.9 \pm 0.7 \mu\text{g}/\text{cm}^2$ in P3), the amount of silver released from P3 coating over the 7 day period was only slightly higher than the other two coatings. For the AgNP-PDA-coated catheter segments (P1, P2, and P3), silver existed as AgNPs in the PDA layer, and the AgNPs have to be oxidized to Ag^+ ions before they diffused out. The PDA layers stabilized the AgNPs and retarded their oxidation.²⁷ Furthermore, the additional PDA layers on top of the AgNP coating served as barriers to inhibit the diffusion process. The Dover™ silver-coated catheter has less silver than the P1 and P2 coated catheters ($10.2 \pm 0.6 \mu\text{g}/\text{cm}^2$ in Dover™), but its release rate was higher in the beginning of test, although it decreased after 3 days. Thus, the phosphate hydrogel coating was not as effective in ensuring a linear release as the P2 and P3 coatings. The silver release rate in sterilized artificial urine may not be a good representation of the actual application conditions. Hence, silver release in artificial urine with inoculated *P. mirabilis* was also investigated, and compared with that in sterilized artificial urine as discussed in Example 10.

Urine contains high concentrations of chloride and phosphate ions ($\sim 126 \text{ mM}$ of Cl^- and $\sim 20 \text{ mM}$ of PO_4^{3-} in the artificial urine used in this study) which may react with silver ions to form insoluble silver salts (e.g. AgCl, silver phosphates). Insoluble salts of $\sim 1\text{-}6 \mu\text{m}$ were formed when the silver-coated catheters (Dover™, P2 and P3) were incubated in artificial urine (Figure S11A). After the insoluble salts were removed using a $0.2 \mu\text{m}$ filter, it was found that $\sim 80\%$ of the silver remained in the filtrate, indicating they are mainly soluble Ag species (Figure S11B). Bacterial assay also confirmed that the filtrate retained $> 99\%$ of the bactericidal efficiency (Figure S11B). It has been reported that the nature of the soluble Ag species in solution varies as a function of Ag and Cl concentrations, and as Cl/Ag ratio increases, $\text{AgCl}_x^{(x-1)-}$ species begin to dominate compared to solid AgCl.²⁸ The amount of silver released from a 1 cm silver-coated catheter segment (Dover™, P2 and P3) into the 2 ml sterilized artificial urine after 24 h ranged from 0.18 to $0.34 \mu\text{g}/\text{ml}$ ($1.7\text{-}3.2 \mu\text{M}$, Figure S11B). The Cl/Ag ratio in the artificial urine was calculated to be $3.9 \times 10^4\text{-}7.4 \times 10^4$, and in this range, the formation of soluble $\text{AgCl}_x^{(x-1)-}$ species can be expected, and they can even be the dominant Ag species.²⁸ Therefore, Ag released from the coatings into urine may exist as Ag^+ , insoluble Ag salts, and soluble $\text{AgCl}_x^{(x-1)-}$ species, and all of these have bactericidal actions.^{28,29}

Example 10**Silver release in artificial urine medium inoculated with *P. mirabilis***

The release of silver from the Dover™ silver-coated catheter, P1, P2 and P3 coated catheter segments was also tested in *P. mirabilis*-inoculated artificial urine medium (10^5 cells/ml), and the results are shown in Figure 7B&C. The Dover™ silver-coated catheter continuously released silver only in the first 5 days. With the drastic decrease in the silver release rate after Day 5, it was not surprising that encrustation of this catheter occurred between Day 6 and 7 (see encrustation assay results in Example 11). This release behaviour is different from that in the sterilized artificial urine condition (Figure 7A), where an almost linear release rate over 7 days was observed. This difference is likely due to the formation of a conditioning layer (containing lysed bacterial cells and medium components) and subsequent encrustation on the coating which obstructed the release of silver (discussed in Example 11). The P1, P2 and P3 coated catheter segments continued to release silver after 7 days, although a gradual decrease in rate was observed for the P1 and P2 coated catheters over this period. Comparing the release rates in the bacteria-infected artificial urine (Figure 7B&C) with those in sterilized artificial urine (Figure 7A), it can be seen that more silver was released from all four catheters in the former over the period before encrustation occurs. The increased elution of silver species may have resulted from their interaction with the sulfide or SH- groups in the medium and/or bacterial membrane, as well as the bio-degradation of the polymeric component of the coating due to bacterial action as discussed in Example 11.

Example 11**Encrustation assay**

To simulate a continuous infection situation, artificial urine inoculated with *P. mirabilis* at a concentration of 10^5 cells/ml was used for the encrustation assay, and this bacteria-inoculated medium was changed daily. This medium was observed to turn turbid within 24 h due to precipitation of crystals as a result of the increase in pH of the medium arising from the hydrolysis of urea by *P. mirabilis*,⁶ as indicated by Sample i in Figure 8A. Figure 8B&C shows the morphology and EDX elemental analysis of the crystals collected by centrifugation from Sample i. As can be seen, the crystals were mainly calcium and magnesium phosphates precipitated from the urine. The results obtained with catheter segments immersed in the *P. mirabilis*-inoculated artificial urine medium on Day 1 and Day 7 are shown in Figure 8A. All artificial urine samples with the silver-coated catheters (Samples ii, iii, iv, v, containing Dover™, P1, P2, P3 catheter segments, respectively)

remained clear on Day 1 with no sign of precipitation. This can be attributed to the bactericidal effect of the released silver species as well as the AgNPs on the catheter coating. However, on Day 7, the artificial urine with the Dover™ silver-coated catheter segment (Sample ii) turned turbid, while the medium with the AgNP-PDA-modified catheter segments (Samples iii, iv, v) remained clear. The average duration before encrustation was observed with the different catheter segments is given in Figure 9. The Dover™ silver-coated catheter inhibited encrustation for < 7 days. With the P1 and P2 coated catheter, the medium remained free of precipitate for 8 and 12 days, respectively. The P3 coated catheter maintained its ability to resist encrustation for as long as 45 days, which is longer than the period required for short-term catheterization (30 days).³⁰

Figure 10A to 10F show the cross-sections of the intraluminal coating of the catheter segments before and after the encrustation test. The Dover™ silver-coated catheter has a silver phosphate hydrogel coating with a thickness of $5.1 \pm 0.4 \mu\text{m}$ (Figure 10A). The P2 and P3 coated catheters have coatings of thickness of $8.6 \pm 0.4 \mu\text{m}$ and $13.1 \pm 1.2 \mu\text{m}$, respectively (Figure 10B&C). The polymer coatings on the P2 and P3 catheter segments became thinner and more porous after 7 and 40 days in the encrustation test (continuous incubation in *P. mirabilis*-inoculated urine medium), respectively (Figure 10E&F). This may be attributed to the release of Ag^+ from AgNPs, as well as the bio-degradation of the polymeric layers. In contrast, these coatings remained relatively intact when incubated in the sterilized artificial urine for the same period of time (Figure S12). For the Dover™ silver-coated catheter, extensive crystal formation on its surface was observed after 7 days in the medium, and the coating could not be differentiated from the crystalline mass (Figure 10D). The SEM image (Figure 10G) and EDX analysis (Figure 10J) also confirmed the extensive precipitation of salts on the Dover™ silver-coated catheter after 7 days. In contrast, the P2 and P3 coated catheter surfaces were relatively free of crystals after 7 and 40 days, respectively (Figure 10H&I). The EDX spectra of the P2 coated catheter surface after 7 days in the medium, and the P3 coated surface after 40 days showed strong Si signals from the underlying silicone substrate (Figure 10K&L), which is consistent with the SEM images showing that these surfaces were relatively free of crystalline precipitate (Figure 10H&I). It should be pointed out that encrustation occurred not because silver was depleted in the catheter coating. From Figure 5 and Figure 7, it can be calculated that only ~14% of the silver in the Dover™ catheter was released before encrustation occurred. For the P3 coated catheter, although the proportion of silver released was higher than that of Dover™ catheter, ~50% of the silver still remained in the coating after 45 days. Thus, the rate of silver release (and not just the amount of silver in the coating) played an important part in inhibiting encrustation.

Since the P3 coating which has two AgNP-PDA bilayers gave significantly much better performance than the P2 coating which has one bilayer (P3 was free of encrustation for 45 days versus P2 for 12 days, Figure 9), a reasonable assumption can be made that if more AgNP-PDA bilayers are included in the coating, the silver release profile can be extended for even longer periods. This is particularly promising for development of urinary catheters and ureteral stents for longer term application.

Example 12

Stability tests

During catheterization or stenting of a patient, the catheter or stent can be expected to be subjected to frictional and bending forces. Therefore, it is important to confirm that the coating on the device surface is stable when subjected to such forces. XPS analysis indicated there was little change in the extraluminal surface composition of the P3 catheter segment before and after the friction and bending tests (Figure S13A). The S 2p and S 2s peaks attributed to the poly(SBMA-co-AAm) coating remained clearly discernible in the XPS wide scan spectra after the stability tests. The surface [Ag]/[C] ratio of the P3 catheter was 0.21% before the tests, and 0.19% and 0.21% after the friction test and bending test, respectively. In addition, similar efficacy in inhibiting *P. mirabilis* biofilm formation was demonstrated by the P3 catheter segments before and after these stability tests (comparing Figure S8 with S13B). Therefore, it can be concluded that the coating on the modified catheter surface was stable and can be expected to withstand friction and bending movements during application.

Example 13

Cytotoxicity assay

The cytotoxicity assay results showed that the catheter extract obtained with an SA/Vol ratio of 1.25 cm²/ml posed minimal cytotoxicity to fibroblasts over 72 h of incubation, but when the SA/Vol ratio was increased to 3 cm²/ml, the medium containing extracts from P3 and Dover™ catheter segments showed some cytotoxicity to the cells (Figure S14A&B). PDA and polySBMA coatings have previously been shown to exhibit minimal cytotoxicity.^{18,31} From the determination of the Ag concentration in the extracting medium obtained with different SA/Vol ratios (Figure S14C), it can be concluded that there was minimal cytotoxic effect (i.e. ≥ 90% cell viability) when the eluted silver concentration in the medium is ≤ 1.5 µg/ml. These results were further confirmed with cell viability tests

using medium containing different concentrations of Ag species from AgNO₃ (Figure S14D).

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Claims

1. A medical device comprising a support substrate having a modified surface, wherein said support substrate comprises silicone, said modified surface having a thickness of 2-20 μm and comprising two or more layers covering all or part of the device surface, said layers comprising: a first layer comprising a sustained release polymer associated with one or more antimicrobial metal ions or nanoparticles, a second layer contacting the first layer and comprising a sustained release polymer and further comprising a biocompatible uppermost layer comprising an anti-fouling polymer, wherein in said biocompatible layer comprises poly(SBMA-co-AAm), wherein, the modified surface inhibits bacterial adhesion and/or biofilm formation and encrustation of the medical device.
2. The device according to claim 1 wherein the device comprises at least one further layer between the second and uppermost layers comprising a sustained release polymer with one or more antimicrobial metal ions or nanoparticles.
3. The device according to claim 1 or 2 wherein said device surface comprises a plurality of first and second layers to provide a multi-layered device surface.
4. The device according to claim 1 wherein the thickness of said modified surface is 10 μm +/- 10%.
5. The device according to any one of claims 1 to 4 wherein said first and second layers comprise the same sustained release polymer.
6. The device according to any one of claims 1 to 4 wherein the first and second layers comprise a different sustained release polymer.
7. The device according to any one of claims 1 to 5 wherein said sustained release polymer is polydopamine, or its functionally equivalent derivatives.
8. The device according to any one of claims 1 to 7 wherein said ion or nanoparticle comprises an antimicrobial metal selected from the group consisting of: silver, gold or copper.
9. The device according to claim 8 wherein said antimicrobial metal ion[s] or nanoparticle[s] comprise silver.

10. The device according to any one of claims 1 to 9 wherein said antimicrobial metal comprises at least 12 μg metal ion[s]/ cm^2 .
11. The device according to claim 10 wherein said antimicrobial metal comprises 12-25 μg metal ion[s]/ cm^2 .
- 5 12. The device according to any one of claims 1 to 11 wherein said support substrate comprises a metal.
13. The device according to claim 12 wherein said support substrate comprises a metal selected from the group: stainless steel, cobalt-chrome (Co-Cr) and titanium and its alloys.
- 10 14. The device according to any one of claims 1 to 13 wherein said modified surface comprises at least one antimicrobial agent wherein said agent is an antibiotic or antimicrobial peptide.
15. The device according to any one of claims 1 to 14 wherein said medical device is a catheter.
- 15 16. The device according to any one of claims 1 to 14 wherein said medical device is a stent.
17. The device according to claim 16 wherein the stent is a ureteral or prostatic stent.
18. The device according to any one of claims 1 to 14 wherein said medical
20 device is a cannula.
19. A method for the fabrication of a substrate surface of a device according to any one of claims 1 to 18 comprising the steps:
- 25 i) contacting all or part of the surface of the substrate with a fluid comprising at least one sustained release polymer to coat the substrate with a first polymeric layer;
- ii) contacting the first polymeric layer with a fluid comprising at least one antimicrobial metal to coat the first polymeric layer with said antimicrobial metal;
- iii) repeating step i) and ii) one or more times;

- iv) contacting the bi-layered or multi-layered coated substrate with a fluid comprising at least one sustained release polymer for controlling the antimicrobial metal release and for anchoring the subsequent anti-fouling layer; and
- 5 v) contacting the coated substrate with a fluid comprising at least one anti-fouling polymer;
- wherein the substrate comprises silicone and a modified surface with a biocompatible uppermost layer having a thickness of 2-20 μm ; and
- wherein the biocompatible upper layer comprises poly(SBMA-co-AAm).
- 10 20. The method according to claim 19 wherein the substrate is immersed in a liquid comprising said sustained release polymer which is subsequently immersed in a liquid comprising said antimicrobial metal.
21. The method according to claim 19 wherein the substrate is contacted with a liquid spray comprising said sustained release polymer which is subsequently
- 15 contacted with an aerosolized fluid comprising said antimicrobial metal.
22. The method according to any one of claims 19 to 21 wherein said sustained release polymer is polydopamine.
23. The method according to any one of claims 19 to 22 wherein said antimicrobial metal is silver.
- 20 24. A device comprising a substrate obtained by the method according to any one of claims 19 to 23, wherein the substrate comprises silicone and a modified surface with a biocompatible uppermost layer having a thickness of 2-20 μm said biocompatible upper layer comprises poly(SBMA-co-AAm); and wherein the modified surface inhibits bacterial adhesion and/or biofilm formation and encrustation of the
- 25 device.
25. The device according to claim 24 wherein said device is selected from the group consisting of: a catheter, stent, or cannula.
26. The device according to claim 25, wherein the stent is ureteral or prostatic stent.