Anti-bacterial, anti-inflammatory and anti-adhesive coatings for urinary catheters

A Thesis

Submitted to the Faculty

of

Drexel University

By

Ce Zhou

in partial fulfillment of the

requirements for the degree

Master of Science in Biomedical Engineering

January 2014

Dedications

This thesis is dedicated to my family and friends who have supported and encouraged me through this entire process. Without their help, I cannot complete this work.

Acknowledgements

I would like to acknowledge my adviser, Dr. Yinghui Zhong, for her guidance on this project, my committee members, Dr. Suresh G. Joshi and Dr. Lin Han, for their advice and guidance in completing this thesis, and my lab mates Zhingling Zhang, Robert Shultz,Camilla Nix, Xiaoyun Niu, Jia Nong, and Zhicheng Wang for their help and advice in completing this project everything they contributed to make it better.

CONTENTS

LIST OF FIGURES

Abstract

Anti-bacterial, anti-inflammatory and anti-adhesive coatings for urinary catheters

Ce Zhou

Patients with permanent injury or severe illness may need a long term use of urinary catheters. Catheter insertion and long-term catheterization carries a significant risk of catheter-associated urinary tract infection (CAUTI) and inflammatory reaction around inner urethra. In this study we developed novel antibacterial, anti-inflammatory, and anti-adhesive coatings based on layer-by-layer (LbL) assembly for effective prevention of infection and inflammation associated with urinary catheters. We tested different base layers to improve the surface wettability and charge of polyvinyl chloride (PVC) urinary catheters for subsequent LbL assembly including different layers of polyethyleneimine (PEI), Dopamine (DA), and alternate adsorption of PEI and polystytene sulfonate (PSS), and found that 10 bilayers of PEI/PSS-coated catheters demonstrated the highest surface wettability and positive charges. We subsequently built a novel anti-bacterial, anti-inflammatory LbL coating impregnated with minocycline hydrochloride (MH), an antibiotic and anti-inflammatory drug on this base layer. MH was incorporated in the LbL assembly based on a novel calcium binding-mediated interactions, which in combination with protective LbL layers, allows sustained release of MH in urine for over 33 days. In particular, initial colonization of three CAUTI related bacterial biofilm, *Escherichia coli (E.coli), Acinetobacter baumannii (A. Baumannii)* and *Staphylococcus aureus (S.aureus)*, was significantly inhibited on catheters coated by MH-releasing LbL coating. Additionally, we developed

an anti-adhesive LbL coating by alternate adsorption of anti-adhesive poly(l-glutamic acid)-grafted-poly(ethylene glycol) (PGA-g-PEG) and gelatin. The adhesion of bovine serum albumin, bacteria, and macrophages on 8 bilayers of PGA-g-PEG/gelatin was greatly reduced.

INTRODUCTION

1. Urinary catheters and CAUTI

Each year, there are more than 5 million patients who need urinary catheters[1]. The urinary catheter system consisted of a tube inserted into the bladder through the urethra to drain urine into a container (Fig.1). Catheter insertion and long-term catheterization carries a significant risk of catheter-associated urinary tract infection (CAUTI). CAUTI is the second most common cause of nosocomial bloodstream infection. Patients requiring. Approximately 25% of hospitalized patients requiring indwelling urinary catheterization for more than 7 days developed nosocomial bacteriuria or candiduria at a daily risk of 5% [2]. Catheter-associated urinary infections (CAUTI) accounts for 30% to 40% of nosocomial infections resulting in morbidity and increased length of hospital stay by 1 to 3.8 days [3]. The primary cause of CAUTI is biofilm formation by pathogens assembled on urinary catheters. The formation of biofilm may increase the resistance to antibiotics. Current methods to prevent biofilm formation are inadequate. Since biofilm plays a major part in the pathogenesis of CAUTI, as well as their high morbidity, mortality, clinical and economic significance, much effort have been made to prevent biofilm formation by modification of catheter surface, such as coating diverse biocompatible materials on the surface on the catheters[4, 5]. Recently, catheters coated with silver alloy have been applied to clinical use, based on the antiseptic ability that silver has exhibited in vitro [4, 6, 7]. However, one silver alloy urinary catheter cost about $6 more than standard catheter [6]. Another

potential concern with the use of silver on catheter coatings is argyrism [8]. An alternative strategy is to use antibiotic-impregnated coatings. Catheters impregnated with a combination of rifampicin and minocycline have been found to be effective in reducing catheter-associated bacteriuria [9]. However, rifampicin is highly toxic [10] which makes it more suitable for treatment, not prevention. Additionally, the widespread use of rifampicin may cause increased rates of drug-resistant tuberculosis [8]. Thus, more economic and safer coatings are required for clinical use.

Another possible consequence that may occur during urinary catheter insertion and long-term use is inflammation, which is caused by bacterial infection and the foreign body reaction[11], resulting in inflammation around inner urethra. This foreign body response is caused by non-specific immune response to implanted foreign materials. It is characterized by the inflammatory cells to the area to destroy or remove the foreign materials. However, since the implants cannot be removed or destroyed, the inflammatory response will persist [12]. Traditional administration of anti-inflammatory agents is often ineffective in treating implant-induced local inflammation

Currently urinary catheters are made of silicone, rubber, latex or polyvinyl chloride (PVC). Urinary catheters with hydrophilic surface coating reduce the risk of biofilm aggregation, as well as protein adsorption and cell adhesion. People with a permanent injury or severe illness may need a long time use of urinary catheters. Short period of catheter usage may not cause a problem, however, long-term use of urinary catheters may cause CAUTI. The pathogenic biofilm can easily formed on the surface of the indwelling urinary catheter due to the moist environment. The indwelling urinary

catheter normally cannot be cleared of a pathogenic biofilm by antibiotics without removing the catheter[4]. As the colonized catheter remains inside the urethra, biofilm-associated bacteria can enter the urine to infect the whole urinary system. Studies have shown that infection occurs within four days when open catheters are used [13]. On one hand, some patients with severe illness or no consciousness cannot avoid from long term use of open catheters, on the other hand, oral or injectable antibiotics and anti-inflammatory drugs often have a low efficiency in finding the target and even cause severe side effects. Sustained local drug delivery system coated on urinary catheters offers a desirable local drug delivery system to effectively inhibit CAUTI.

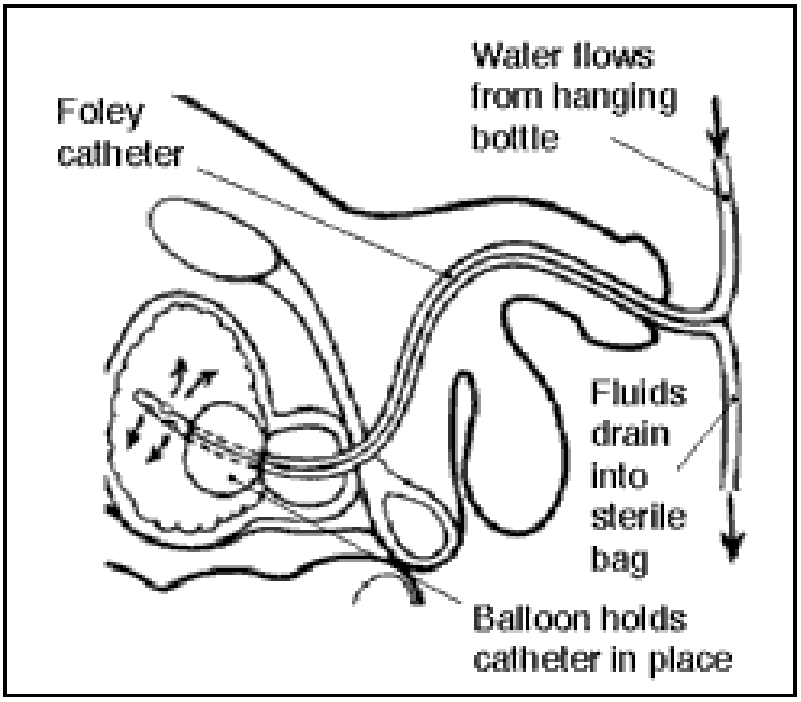

Fig.1 Use of urinary catheters

2. Loading drug with electrostatic layer-by-layer assembly

Hydrophilic coatings have been shown to minimize nonspecific protein adsorption and inflammatory cell adhesion that would exacerbate the foreign body response [14].

As free energy is minimized when proteins stick to surfaces compared to being surrounded by water molecules, hydrophilic surfaces have a lower protein adsorption. Thus, the ability to coat a hydrophilic film on hydrophobic catheter surface is a crucial step for the following study of cell/bacterial/protein –biomaterial interactions. Several surface modifications have been reported, including plasma modification[15], and chemical modification[16]. Plasma modification brings in a high-quality thin film, but inner surface of tubing cannot be accessed easily by this method. Electrostatic layer-by-layer (LbL) method, however, is based on alternate adsorption of oppositely charged components onto solid substrates, has been developed to coat nanometer-thick organized films on any surface [17-19].

Minocycline Hydrochloride (MH) is a broad-spectrum tetracycline antibiotic and long-acting effective anti-inflammatory drug that is widely used to treat infection and inflammation [20], making it a promising drug candidate to treat implant-associated infection and inflammation by layer-by-layer (LbL) local delivery. Our lab has previously developed a novel hydrophilic multilayer thin film coating capable of sustained release of MH in Hank's Balanced Salt Solution (HBSS) for over 35 days based on a novel calcium binding-mediated drug delivery mechanism. MH can chelate divalent metal ions such as Ca^{2+} and Mg^{2+}, without affecting its biological activities

[21]. Dextran sulfate (DS) is a biocompatible and biodegradable polysaccharide that also has a high binding affinity for Ca^{2+} due to its numerous negatively charged sulfate groups [22]. Utilizing this property, our lab used Ca^{2+} as the linker to attach MH to DS. We further found that DS can form electrostatic layer-by-layer (LbL) with gelatin type A (GA), a positively charged biocompatible, biodegradable natural polymer derived from collagen. Based on our lab's previous work, multilayers of DS-Ca^{2+}-MH conjugate/GA were successfully constructed to form hydrophilic coatings. However, MH release from 20 trilayers of LbL films only lasted for 18 days in urine. To prolong MH release, we added alginate sulfate (AS) and glycol-chitosan (GC) to the trilayers system (Fig.2A) to server as barriers to impede MH release. MH release from 20 pentalayer of LbL film into urine was increased to 33 days using this strategy.

Successful deposition of the hydrophilic MH-releasing LbL coating onto a urinary catheter requires a hydrophic and charged surface. However, the PVC urinary catheter used in this study is hydrophobic and neutrally charged, which doesn't support charged hydrophilic polymer adhesion. Thus, the catheter surface needs to be modified to create a charged and hydrophilic surface for subsequent LbL assembly. Polyethyleneimine (PEI) is a positively charged polymer due to the numerous amine groups [5]. Polystyrene sulfonate (PSS) is a negatively charged polymer because of the sulfonic acid or sulfonate side groups [5]. Multilayer of alternating cationic PEI and anionic PSS has been used as the base layer for LbL assembly [5]. PEI/PSS ending by PEI can potentially improve the wettability of catheter surface and make it positively charged by providing the surface with amine groups, making it a good candidate for a base layer.

Dopamine (DA), a small molecule compound that contains mussel adhesive functional groups, can form both strong covalent and no-covalent interactions with diverse substrates [23]. DA also contains numerous amine groups which provide coated surface with positive charges.

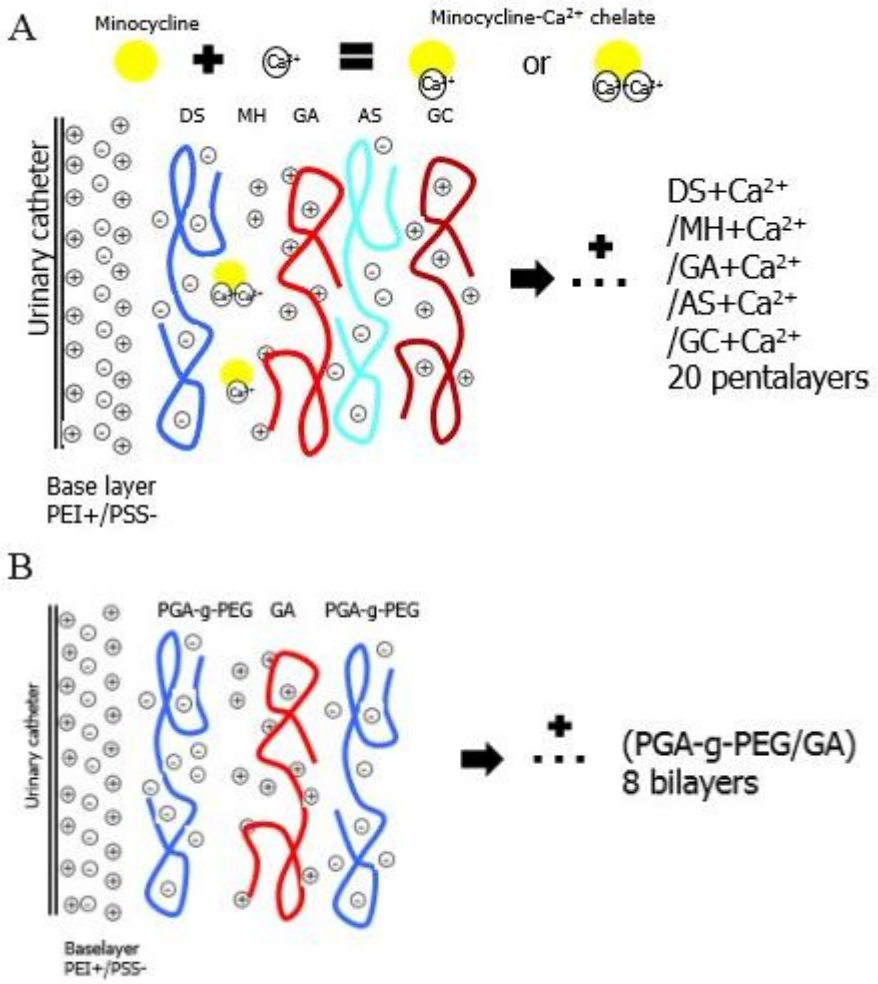

Fig 2 (A) Scheme of MH pentalayers LbL assembly. (B) Scheme of anti-adhesive LbL assembly.

3. Role of polyethylene glycol (PEG) in anti-protein adsorption, anti-cell adhesion and anti-bacterial adhesion

Proteins are prone to adhere to the surface of medical devices due to reduced free energy [24]. Adhered proteins play an essential role in the initial adhesion of bacteria and inflammatory cells on a biomaterial surface [25], the first step in biofilm formation and foreign body response. Infection and inflammation are common causes for the failure of many medical implants [26]. Tremendous research effort has been made to develop strategies to resist protein adsorption and subsequent bacteria and cellular adhesion on medical devices [27, 28]. Surface modification to combat these undesirable interactions are usually chosen based on the application of the medical devices and underlying material. Our molecule that has shown great "non-fouling" potential is polyethylene glycol (PEG) [25]. PEG is a biocompatible non-ionic polymer that resists protein adsorption and cell adhesion due to its strong affinity for water molecules [29]. However, it cannot be incorporated into electrostatic LbL due to lack of charges. One study reported coupling of PEG to poly(L-glutamic acid)(PGA) to obtain negatively charged poly(l-glutamic acid)-grafted-poly(ethylene glycol) (PGA-g-PEG) [29]. In the same study a multilayer of poly(l-lysine) (PLL)/PGA-g-PEG was built and demonstrated to reduce both protein and bacterial adhesion. However, while immobilized PLL has been commonly used for cell culture, free PLL has been reported to have cytotoxicity [30]. Since PGA is degradable, PLL can be released from the multilayer film and becomes cytotoxic. In the present study we tested replacing PLL with gelatin type A (GA) or chi to improve the safety of the anti-adhesive LbL film. We

found that multilayers of GA/PGA-g-PEG demonstrated higher anti-adhesive capability than chi/PGA-g-PEG. *Escherichia coli (E.coli), Acinetobacter baumannii (A. Baumannii)* and *Staphylococcus aureus (S.aureus)* are the common pathogens that cause CAUTI [31]. 8 bilayers of GA/PGA-g-PEG (Fig.2B) deposited urinary catheters significantly inhibited protein adsorption, biofilm formation by all three bacteria species, and macrophage adhesion. This anti-adhesive coating, in combination with the MH-releasing coating, is highly promising to effectively prevent infection and inflammation associated with indwelling urinary catheters.

MATEIRALS AND METHODS

1. Materials

Dextran Sulfate (DS), Minocycline Hydrochloride (MH), Gelatin type A (GA), Alginate Sulfate (AS), Glyco-Chitosan (GC), Polyethelineimine (PEI), Polystyrene Sulfonate (PSS), and Dopamine (DA) were purchased from Sigma-Aldrich. Paraformaldehyde (PFA) 16% solution was purchased from Electron Microscopy Sciences. Trypone Soya Broth (TSB) was purchase from Oxoid. Nutrient Agar was purchased from Remel. 4',6-diamidino-2-phenylindole (DAPI) was purchased from Invitrogen. PVC catheters were obtained from C.R. Bard, Inc (Covington, GA). HPLC-grade methanol, acetonitrile, phosphate were obtained from Sigma-Aldrich (St. Louis, MO, USA). All the other reagents were at their highest purity and water was deionized water (DI). PVC latex-free urethral catheters were from Bard.

RAW264.7 murine macrophages were provided by Dr. Narayan Avadhani, University of Pennsylvania. Urine was collected from healthy volunteers from Drexel University and sterilized by 0.22μm filter. *Escherichia coli* (ATCC 25922), *Acinetobacter baumannii* (ATCC 19606), and *Staphylococcus aureus* (ATCC 25923) strains were purchased from American Type Culture Collection (ATCC, Manassas, VA). A multi-drug resistance *Acinetobacter baumannii* clinical isolate (referred as A. baumannii #22) was locally isolated from a hospitalized patient having invasive device-

associated infection following a protocol approved by the Institutional Review Board of Drexel University.

2. Preparation of PEG-PGA conjugate

2.1 PEG-NH2 conjugate

200mg PEG was dissolved in 10 mL dimethylformamide (DMF). 323mg 1,1'-carbonyldiimidazole was mixed in the PEG solution. The reaction was allowed to proceed with stirring for 2 h at room temperature. Subsequently, 5 mL of ethylenediamine was added and the reaction was allowed to proceed with stirring for 24 h at room temperature. 10mL deionized (DI) water was added to stop the reaction and the reaction mixture was dialyzed against DI water for at least 18 h with at least 3 buffer changes. The product was lyophilized and stored at -20℃.

2.2 Graft PEG onto PGA

40 mg of PGA, 95.2 mg of PEG-NH_2 and 2 mg of NHS were dissolved in 2ml of buffer, 100mM sodium tetraborate (pH=8.5). EDC (14.6 mg) was dissolved in the mixture with stirring. The reaction was allowed to proceed at room temperature for 6 h. After filtration, the reaction mixture was dialyzed for 24 h, first against phosphate buffer (0.1 M, pH=7.4, Na_2HPO_4/NaH_2PO_4) and subsequently against DI water. PEG-PGA was lyophilized and stored at −20°C [29].

3. Catheter Surface Modification

3.1 Base layer

Solutions of PEI and PSS were prepared in $CaCl_2$ solution with a concentration of 0.8mg/mL in deionized (DI) water. The urinary catheters were coated with 10 layers of polyethyleneimine (PEI) incorporated with polystyrene sulfonate (PSS) or 10 layers of Dopamine as base layers

3.2 LbL assembly of MH-releasing multilayer film

MH-releasing multilayer films were deposited on UV-transparent 96 well plates for characterization of MH incorporation and film growth by UV absorption, or on PVC catheters for release study. 1 mg/ml solutions of DS, MH, GA, AS and GC were prepared in 0.8 mg/mL $CaCl_2$ in deionized (DI) water solution. The substrates The substrates were first coated with 10 bilayers of PEI/PSS as an initiating positively charged base layer, followed by alternating immersion in solutions of DS, MH, GA, AS and GC for 10 min. The excess molecules were removed by rinsing the substrates with DI water for 1 min between each step.

3.3 LbL assembly of anti-adhesive multilayer film

Anti-adhesive multilayer films were deposited on PVC catheters. 1 mg/ml solutions of PGA-g-PEG and GA were prepared in 0.8 mg/mL $CaCl_2$ in DI water solution. The substrates were first coated with 10 bilayers of PEI/PSS as an initiating

positively charged base layer, followed by alternating immersion in solutions of PGA-g-PEG and GA for 10 min. The excess molecules were removed by rinsing the substrates with DI water for 1 min between each step.

4. Surface Characterization

4.1 Measurement of contact angle

Advancing contact angle measurements were performed on bare catheters and coated catheters via the sessile drop technique[32] using goniometer(Dataphysics). Each data point was tested with approximately 1ul of double-distilled deionized water, based on 3 contact angle measurements at 3 different positions on the catheter specimen.

4.2 Measurement of surface density of amino groups

In order to determine how many amino groups were there on the surface of modified catheters, sulfo-SDTB was employed. The reaction between sulfo-SDTB and amino groups on the modified catheters results in stable bonds at alkaline conditions. 1.5mg sulfo-SDTB was dissolved in 0.5mL DMF, and diluted to 25mL in 50mM sodium bicarbonate buffer (PH=8.5). This solution is added to each well and incubated for 1h, and then use sodium bicarbonate buffer to wash three times. Finally 1mL 35% perchloric acid is added to each well, and absorbance is measure after 8~10 minutes. In addition, the absorbance of 1mL 35% perchloric acid in clean wells is also measured as a background. The amine group concentration was determined by measuring the absorption intensity at 498 nm.

4.3 Thickness measurement

The thickness of LbL films deposited on PVC catheters was measured at dry state using an optical profilometer (Zygo).

5. In vitro MH release in urine

LbL films were incubated in sterile urine at 37 °C for quantification of MH release. Every 24 h, the release medium was removed and replaced with fresh urine. The amount of MH released at each time point was determined and integrated by Waters 1525 high-performance liquid chromatography (HPLC).

5.1 Equipment and chromatographic conditions

The HPLC device was a Waters 1525 binary HPLC pump with Waters 2489 UV/Visible detector and a 20 µl injection loop. The system was equipped with a Waters C18(3.5µm，4.6*75mm) analytical column. The column was heated to 30℃ with a thermostat system. We chose Phosphate buffer (25mM, pH=3), methanol and acetonitrile as mobile phase with a ratio of 91:6:3. Samples collected from daily MH release of pentalayers were filtered with 0.22µm before injected. The UV/Visible detector was set at 245nm wavelength.

6. Bacterial adhesion study

6.1 Bacterial culture

E.coli, A.Baumannii and *S. aureus* were cultured overnight in agar plates for 24 h

at 37℃. 10ul of bacteria were added into 10 mL Trypone Soya Broth (TSB) for *E.coli*, *A.Baumannii* and *S. aureus*. These initial cultures were incubated at 37℃ for 24 h. After that, 100uL of cultured bacteria suspension was transferred into 10mL TSB medium with additional 100uL 50% w/v glucose for seeding. Finally, bacteria were seeded onto catheters in 48-well plate, 500ul per well.

6.2 Bacterial adhesion

The growth of *E.coli*, *A.Baumannii* and *S. aureus* were observed on bare catheters and on anti-adhesion modified catheters coated by $(PEI/PSS)_{10}$-$(PGA\text{-}g\text{-}PEG/GA)_8$. 100uL was transferred into 10mL TSB medium with additional 100uL 50% w/v glucose for seeding. Bacteria were seeded onto catheters in 48-well plate, 500uL per well, and incubated overnight for 24 h at 37℃. The bare and anti-adhesion modified catheters were washed thoroughly with sterile PBS before XTT assay and morphologic imaging.

6.3 XTT assay

XTT was re-suspended from stock solution. Menadione, a catalyst that expedites the XTT reaction, was also added. 5% XTT solution and 1.5% menadione were mixed in to sterile 1x PBS. Each well (48-well plate) will need 400uL mixed solution. Wash the wells with sterile 1x PBS once, then add 500uL mixed solution into each well. Incubate the plate for 2 hours. Fluorescence was measured via microplate reader (Tecan) at 492nm. Data was adjusted by XTT control and sterile 1x PBS negative control.

6.4 DAPI staining and imaging

Remove the media from the wells. Add sterile 1x PBS to rinse the well. Dilute DAPI stock in sterile 1x PBS at the ratio of 1:400. Remove 1x PBS from the wells, add 400uL DAPI solution into each well (48-well plate). Incubate the plate for 30 min, followed by washing by sterile 1x PBS for 3 times. Take fluorescence imaging. Blank controls were run simultaneously during each experiment.

7. Cell adhesion study

7.1 Cell adhesion culture

RAW 264.7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, in a humidified atmosphere with 5% CO_2 at 37 ℃. To maintain the cells, they were normally passed at a ratio of 1: 10 every three days.

7.2 Staining and counting

Pieces of sterile urinary catheters were sterilized for 1 h by UV irradiation before placing them into a 48-well culture plate for cell culture. Subsequently, RAW 264.7 were seeded onto the catheters into the wells at a density of 15×10^4 cells /mL and incubated for 24 h. Catheters were then rinsed thoroughly by PBS twice. Then use immerse catheters into DAPI stain for 15 min. Wash the catheters with PBS for 3 times

before fluorescent imaging. Blank controls were run simultaneously during each experiment. Counting cells by Image J. 3 views per replicate, 3 replicates per treatment.

8. Protein absorption study

Catheters were put into 48-well plate and immersed by FITC-labeled bovine serum albumin (BSA) at a concentration of 200 mg / L which is a risk level indicating a possible kidney damage, at 37 ℃ for 24h. Subsequently, catheters were thoroughly rinsed with PBS for three times before fluorescent imaging. Blank controls were run simultaneously during each experiment. Data for each condition were pooled, and data were analyzed for statistical significance.

9. Microscopy and Image analysis

Cells were counted by Image J 1.46r. SPSS 20.0 was employed for data statistical analysis.

10. Statistical analysis

Two-tailed t test was performed to investigate the significance between the contact angles, amino groups of each base layer, compared to bare catheter and daily MH release between trilayers and pentalayers. Kruskal Wallis test was performed to determine the significance of the amount of each bacterial colony, macrophages density and protein fluorescent absorption on catheters. A p value < 0.05 was considered significant.

RESULTS AND DISCUSSIONS

1. Surface Characterization

1.1 Measurement of contact angle

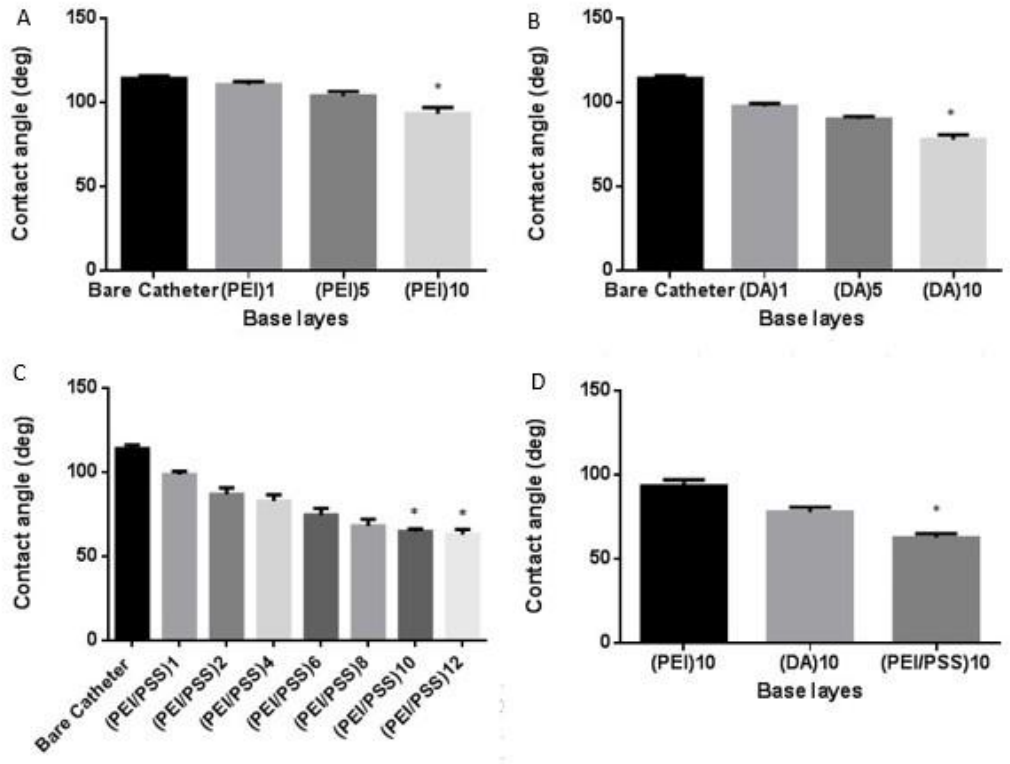

Fig. 3. (A) Contact angle measurement of PEI as base layers with 1, 5, 10 layers. Contact angle of bare catheter is also shown in the figure. *, $P<0.05$, compared to $(PEI)_5$ (B) Contact angle measurement of PEI as base layers with 1, 5, 10 layers. *, $P<0.05$, compared to $(DA)_5$ (C) Contact angle measurement of PEI/PSS as base layers with 1, 2, 4, 6, 8, 10 and 12 layers. Contact angle of bare catheter is also shown in the figure. *, $P<0.05$, compared to $(PEI/PSS)_6$. (D) Contact angle of $(PEI)_{10}$, $(PEI/PSS)_{10}$, and $(DA)_{10}$. *, $P<0.05$, compared to $(PEI)_{10}$ and $(DA)_{10}$. Data shown are average ±SD (n = 6).

Fig. 3 shows water contact angle of PVC catheter modified with different base layers. Bare catheter was hydrophobic with a contact angle of 114.2 ± 1.6°. Fig. 3A shows that water contact angle was reduced with increasing number of PEI layers. Surface modified with $(PEI)_{10}$ had significantly lower contact angle than the others. Likewise, increasing the number of DA layers resulted in reduced contact angle (Fig 3B), and 10 layers of DA had the lowest contact angle. Fig. 3C shows that the contact angle was reduced as the number of PEI/PSS bilayer was increased. The contact angle of $(PEI/PSS)_6$ was significantly higher than that of $(PEI/PSS)_{10}$. However, there was no significant difference between the contact angle of $(PEI/PSS)_{10}$ and $(PEI/PSS)_{12}$, indicating that increasing the number of bilayers beyond 10 will not further improve the surface wettability. Fig. 3D shows that the contact angle of $(PEI/PSS)_{10}$ was significantly lower than that of $(PEI)_{10}$ and $(DA)_{10}$, indicating that it is the most hydrophilic base layer for PVC catheters. Thus, we chose $(PEI/PSS)_{10}$ as the base layer for the subsequent LbL assembly.

1.2 Measurement of amine group surface density

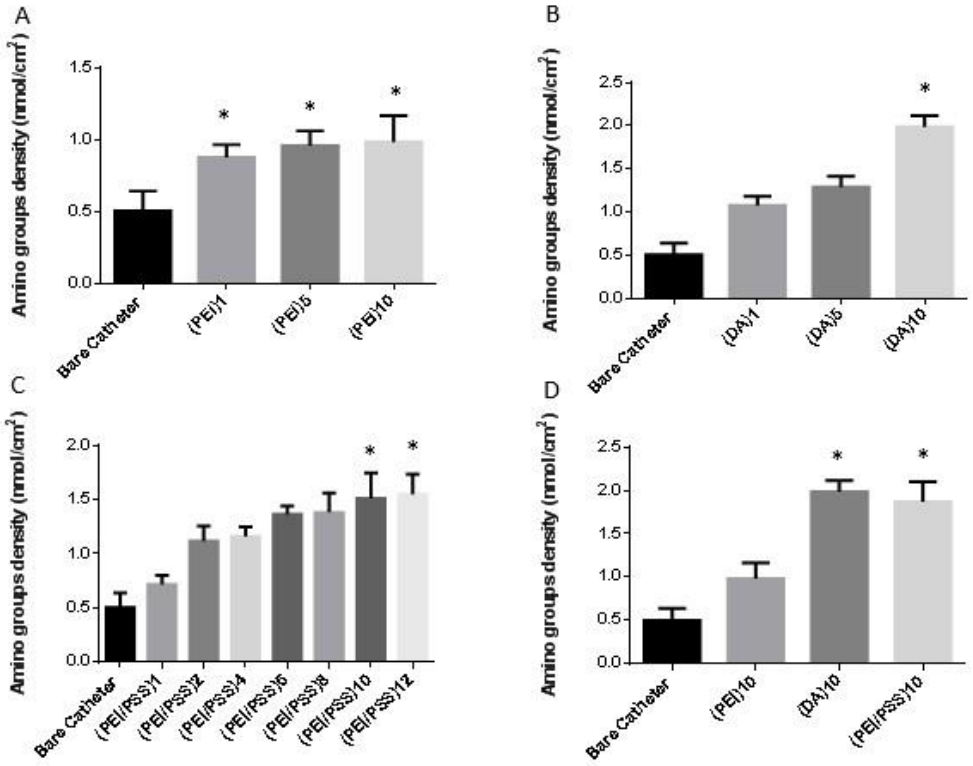

Fig. 4. Measurement of amine group surface density. (A) Sulfo-SDTB measurement of PEI as base layer with 1, 5 and 10 bilayers. *, P<0.05, compared to bare catheter. (B) Sulfo-SDTB measurement of DA as base layer with 1, 5, and 10 bilayers. *, P<0.05, compared to $(DA)_5$. (C) Sulfo-SDTB measurement of PEI/PSS as base layer 1, 2, 4, 6, 8, 10 and 12 layers. *, P<0.05, compared to $(PEI/PSS)_8$. (D) Amino groups density of $(PEI)_{10}$, $(PEI/PSS)_{10}$, and $(DA)_{10}$. *, P<0.05, compared with $(PEI)_{10}$. Data shown are average ±SD (n = 3)

As DS is negatively charged, a positively charged base layer is required for LbL assembly. We measured the surface density of positively charged amino groups on different base layers. More amine groups will increase the base layer's binding affinity for DS. , as well as the charge. Fig. 4A and B shows that the surface density of amine

groups are similar for 1, 5 and 10 monolayer of PEI, while $(DA)_{10}$ significantly increased amino group density than $(DA)_1$ and $(DA)_5$. For PEI/PSS, amino group density increased with the number of bilayers till 10, beyond that the increase in density was not significant (Fig 4C). Fig. 4D shows that $(PEI)_{10}$ has significantly lower anime group density compared with $(DA)_{10}$ and $(PEI/PSS)_{10}$, whereas that of the latter two base layers are significantly different. Combined with the contact angle measurement results, $(PEI/PSS)_{10}$ is the optimal base layer with good hydrophilic and charge density (small contact angle and high amino groups density).

2. Thickness measurement

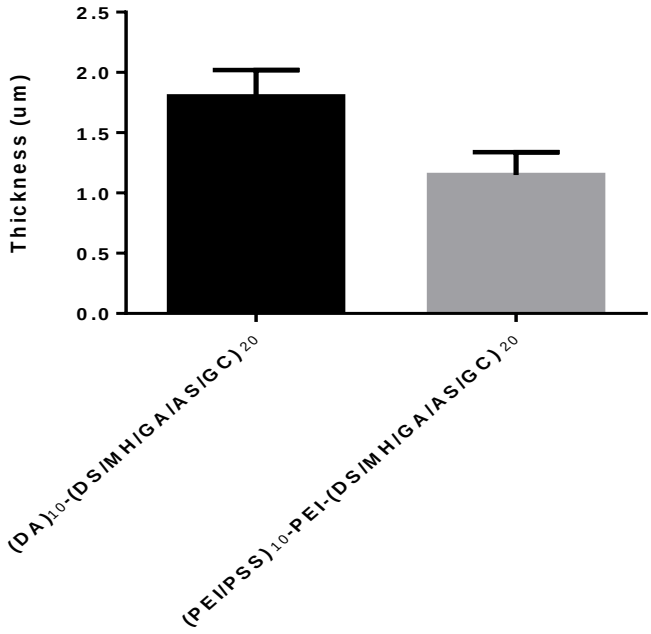

Fig.5 Thickness of and $(PEI/PSS)_{10}$-PEI-$(DS/MH/GA/AS/GC)_{20}$ and $(DA)_{10}$-$(DS/MH/GA/AS/GC)_{20}$. *, $P<0.05$ compared to $(DA)_{10}$-$(DS/MH/GA/AS/GC)_{20}$. Data shown are average ±SD (n = 3)

Fig. 5 shows that the thickness of 20 pentalayers of MH-releasing film with base layer of $(PEI/PSS)_{10}$ and $(DA)_{10}$ is 1.14 ±0.19 and 1.81 ±0.22 μm, respectively. Studies have shown that the thickness of one layer of PEI/PSS and DA are 3 and 10 nm[5, 23], which may contribute to the difference in the total coating thickness.

3. LbL multilayer film growth and characterization

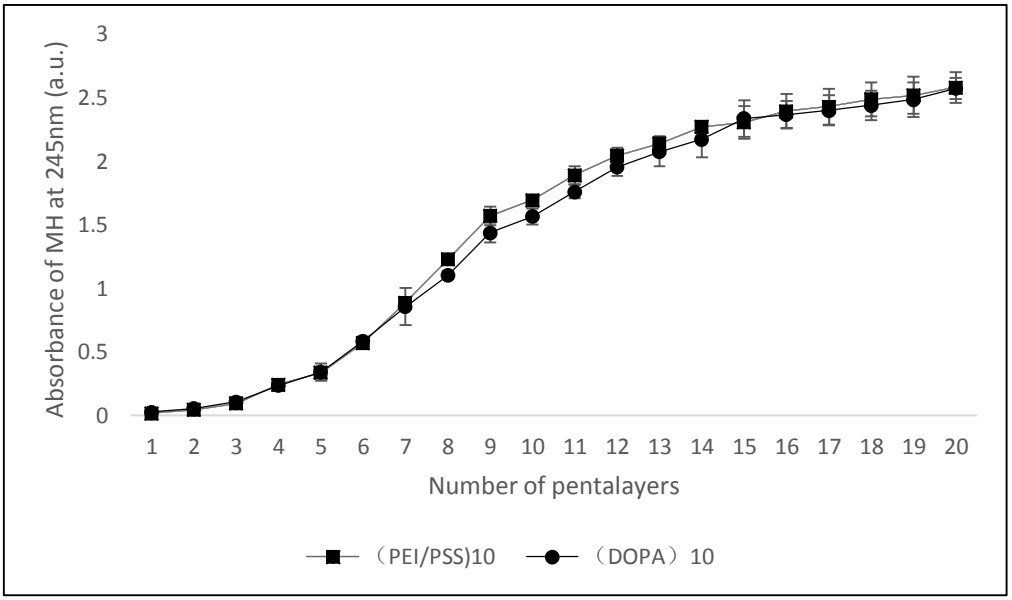

Fig. 6 LbL multilayer film growth and characterization. Data shown are average ±SD (n = 3)

Fig. 6 shows that the UV absorbance of MH increased with the number of pentalayers, indicating successful LbL assembly.

4. MH release in urine

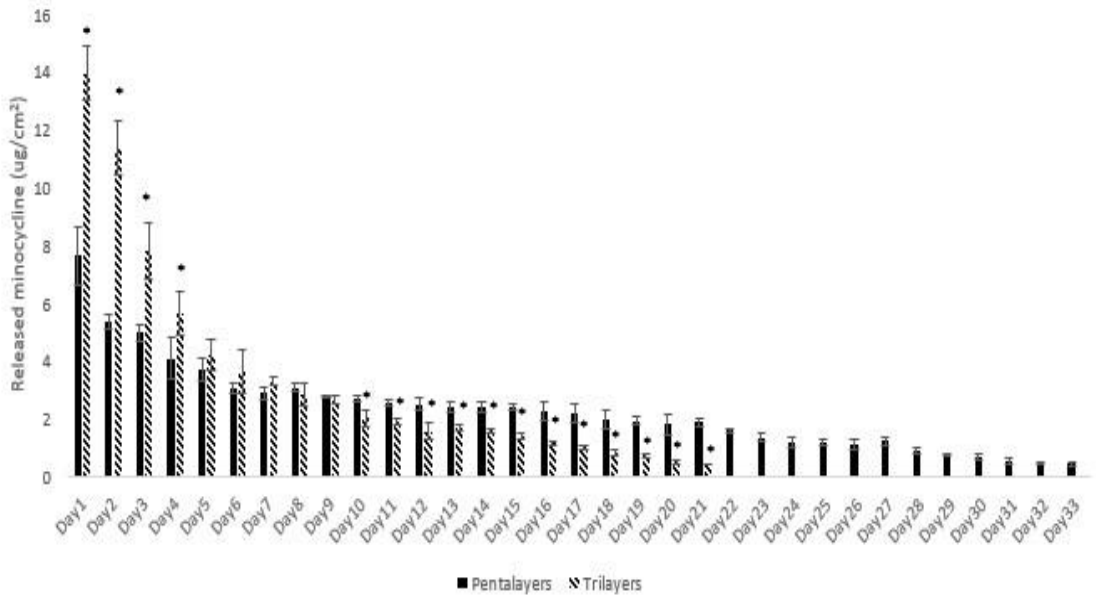

Fig. 7 MH release in urine. Data shown are average ± SD (n=3). *, $P<0.05$ compared to daily MH release of pentalayers coating.

Our lab has previously shown that MH release from PEI-$(DS/MH/GA)_8$ deposited on UV plates lasted for over 35 days in HBSS. However, after we changed the substrates from plates to catheters, the duration of release was drastically reduced to 6 days. When we increased the number of trilayers containing MH from 8 to 20, MH release lasted for 15 days. Although the duration of drug release was prolonged, it was not enough for long-term application. When we changed the base layer from $(PEI)_1$ to $(PEI/PSS)_{10}$ to improve the wettability and increase surface charge of the catheters, MH release lasted for 31 days in HBSS. However, the release only lasted for 21 days after we changed the release medium from HBSS to urine. Molecules in the urine and the slightly acidic pH (7.0) may affect the binding affinity of MH to the coating polymers and hence, the release rate. We added AS/GC on top of each trilayers to protect tgeh

binding of MH to the coating polymers. With the optimized coating design MH release in urine for 33 days was accomplished. Moreover, with this new design the initial burst release was greatly reduced compared with the trilayer design without AS/GC protective layers.

5. Anti-bacterial potency of released MH

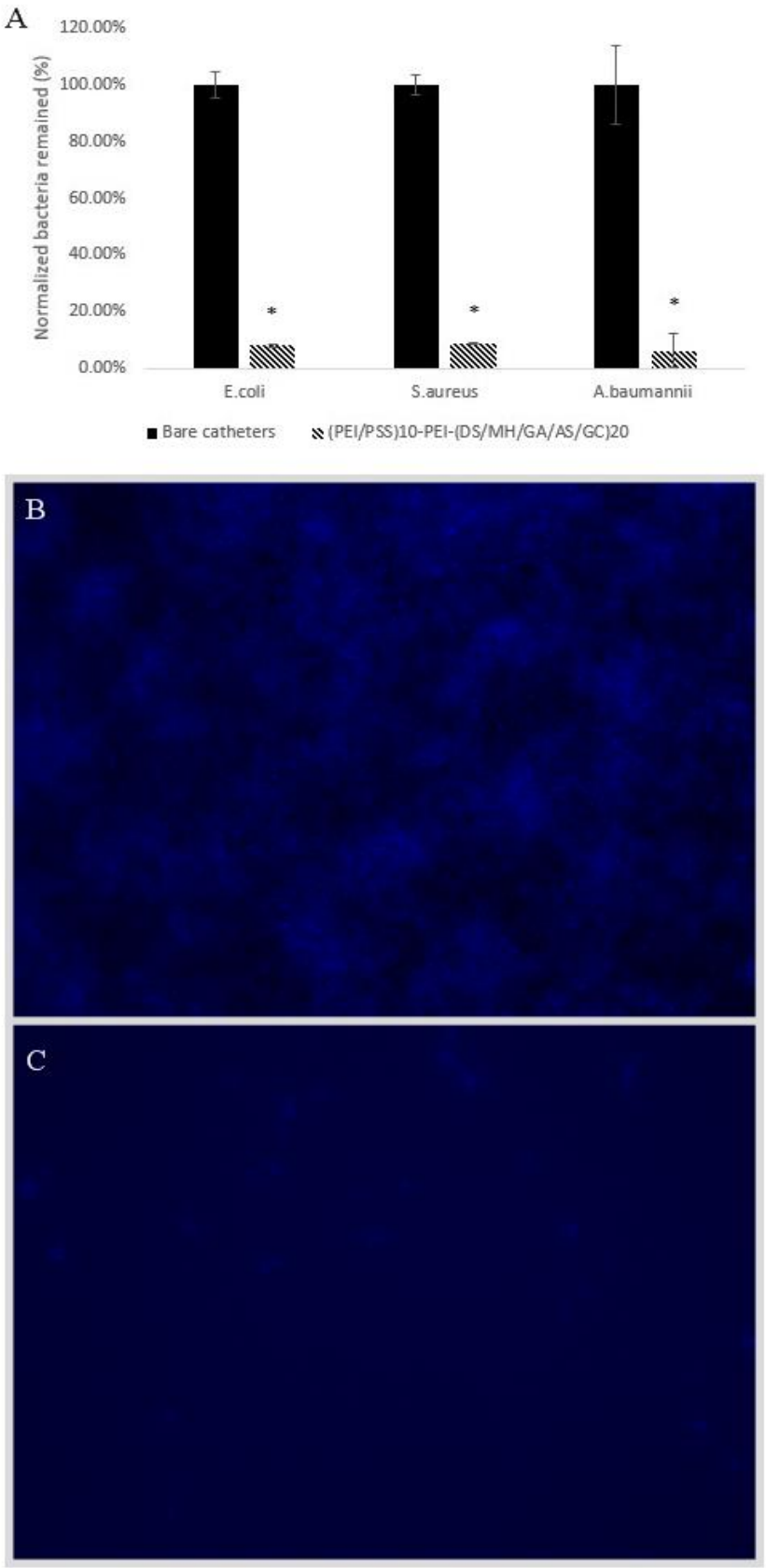

Fig. 8 Anti-bacterial potency of released MH. (A) XTT assay to quantify surviving bacteria (*E.coli, S.aureus* and *A.baumannii*) on bare catheters and $(PEI/PSS)_{10}$-PEI-$(DS/MH/GA/AS/GC)_{20}$ coated catheters. * $P<0.05$ compared to bare catheters. Data shown are average ±SD (n = 4). Fluorescent images of *E.coli* cultured on (B) bare catheters, and (C) $(PEI/PSS)_{10}$-PEI-$(DS/MH/GA/AS/GC)_{20}$ coated catheters after 24h culture. The cells were stained with 4',6-diamidino-2-phenylindole (DAPI).

XTT assay shows that $(PEI/PSS)_{10}$-PEI-$(DS/MH/GA/AS/GC)_{20}$ significantly inhibited biofilm formation by all three bacteria species (Fig. 8A). The percentage of reduction for *E.coli, S.aureus* and *A.baumannii* is 91.9 ±3.7%, 91.2 ±3.2% and 93.8 ± 6.1%, respectively. DAPI staining was used to detect the DNA of bacteria. As shown in Fig. 8B, *E.coli* formed biofilm on uncoated catheters, whereas the number of bacteria was greatly reduced on the coated catheters, confirming the result from XTT assay.

6. Protein adsorption study

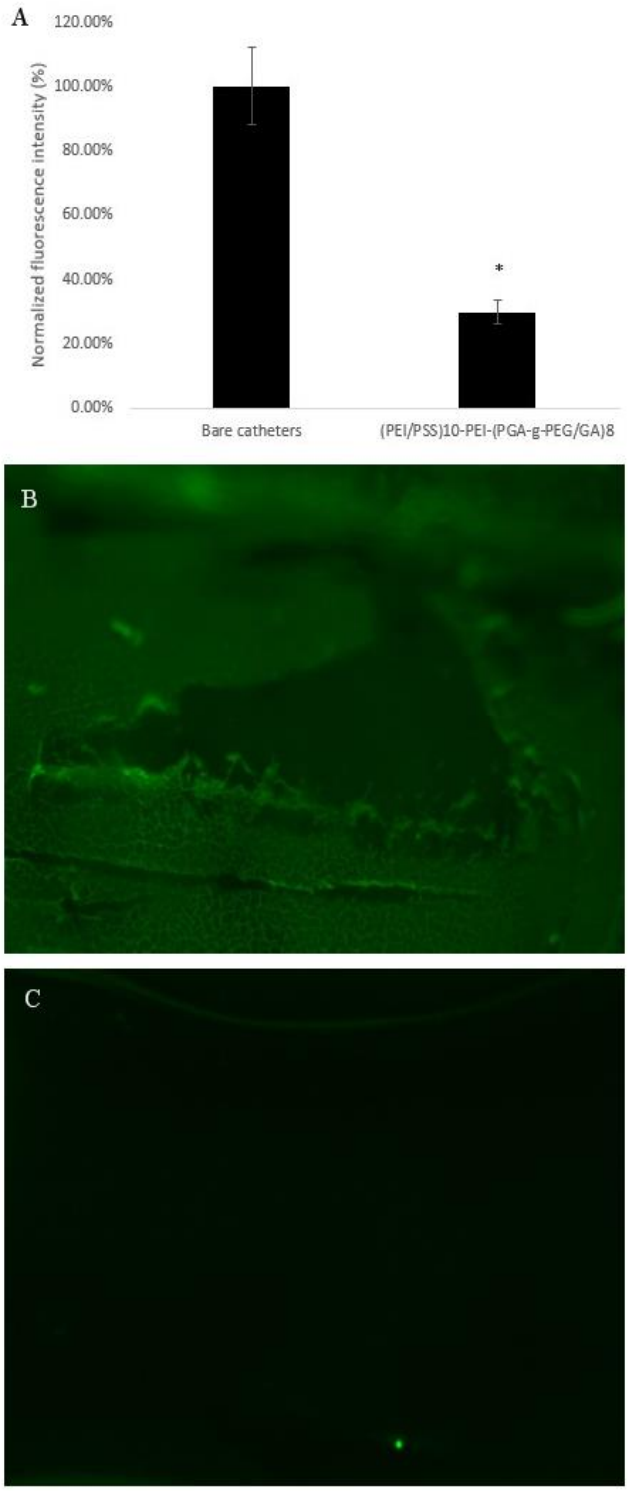

Fig. 9 Protein adsorption study. (A) Fluorescence intensity of bovine serum albumin (BSA) on bare catheters and $(PEI/PSS)_{10}$-PEI-$(PGA\text{-}g\text{-}PEG/GA)_8$ coated catheters. *,

$P<0.05$ compared to bare catheters. Data shown are average ±SD (n = 3). (B) FITC-BSA on bare catheter. (C) FITC-BSA on anti-adhesive catheter.

The fluorescent intensity, as an indicator of BSA adsorption on $(PEI/PSS)_{10}$-PEI-$(PGA\text{-}g\text{-}PEG/GA)_8$ coated catheters were reduced by 70.1 ±29.8%. Compared with the bare catheters, coated catheters had significantly less BSA adsorption after 24h exposure ($P<0.01$). As proteins play an important role in mediating initial cellular and bacterial adhesion onto material surface [33], the anti-adhesive coating developed in this study may further contribute to inhibition of infection and inflammation.

7. Bacteria adhesion study

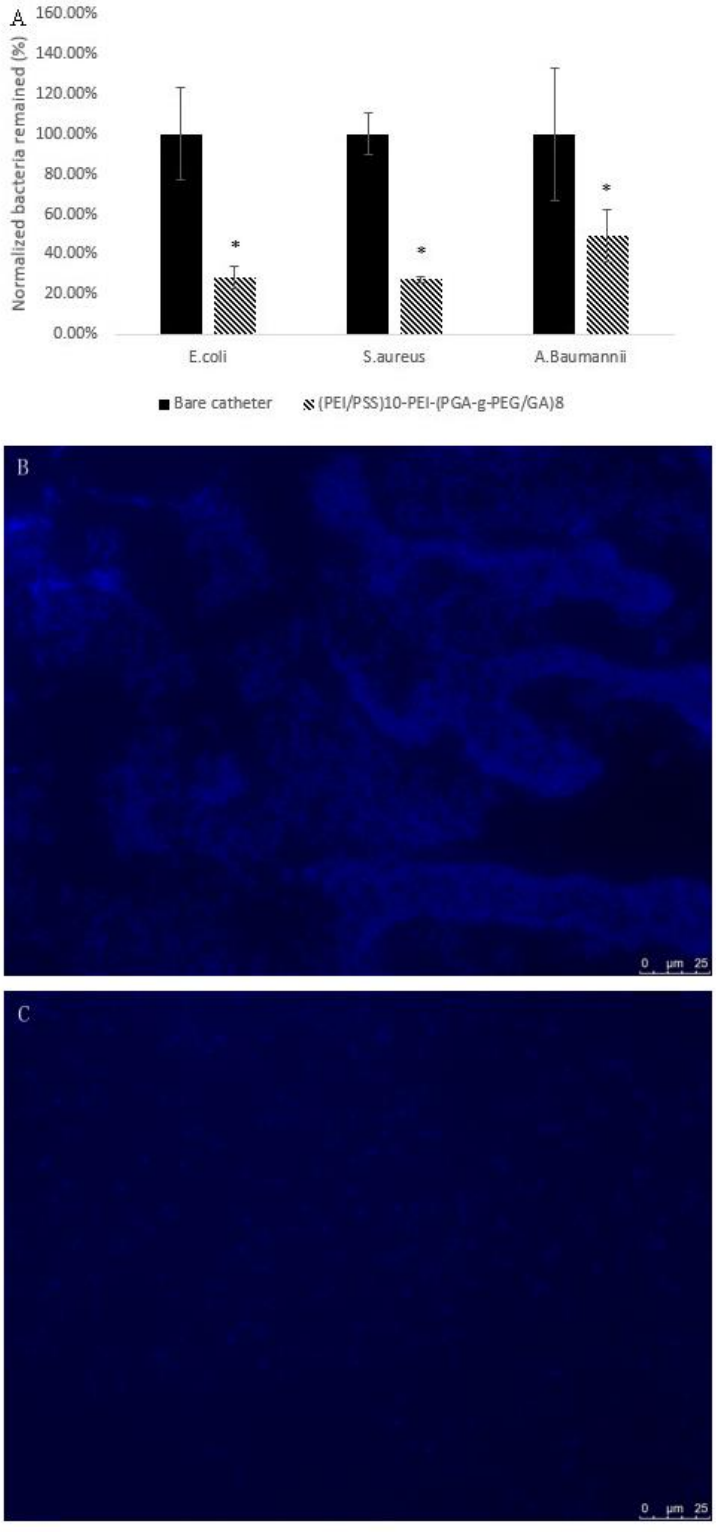

Fig. 10 Bacteria adhesion study. (A) XTT assay to quantify bacteria adhesion (*E.coli*, *S.aureus* and *A.baumannii*) on bare catheters and $(PEI/PSS)_{10}$-PEI-$(PGA\text{-}g\text{-}PEG/GA)_8$

coated catheters. *, $P<0.05$ compared to bare catheters. Data shown are average ±SD (n = 4). (B) *E.Coli* adhesion on bare catheters after 24h culture. (C) *E.Coli* adhesion on $(PEI/PSS)_{10}$-PEI-$(PGA\text{-}g\text{-}PEG/GA)_8$ coated catheters after 24h culture.

XTT assay shows that the number surviving *E.Coli*, a major pathogen causing catheter associated urinary tract infection[31], on $(PEI/PSS)_{10}$-PEI-$(PGA\text{-}g\text{-}PEG/GA)_8$ coated catheters was reduced by 71.5 ± 5.3% compared to the control group. The numbers of *A.Baumannii* and *S.aureus* on coated catheters were reduced by 50.5 ±33.0% and 72.5 ±10.4%, repectively.

Most of the bacterial surfaces have hydrophobic regions[34, 35]. Hydrophobic interaction seems to be essential for bacterial adhesion[36]. Therefore, the anti-adhevie property of our coating could be attributed to the strong hydrophilic PGA-g-PEG chains.

8. Macrophage adhesion study

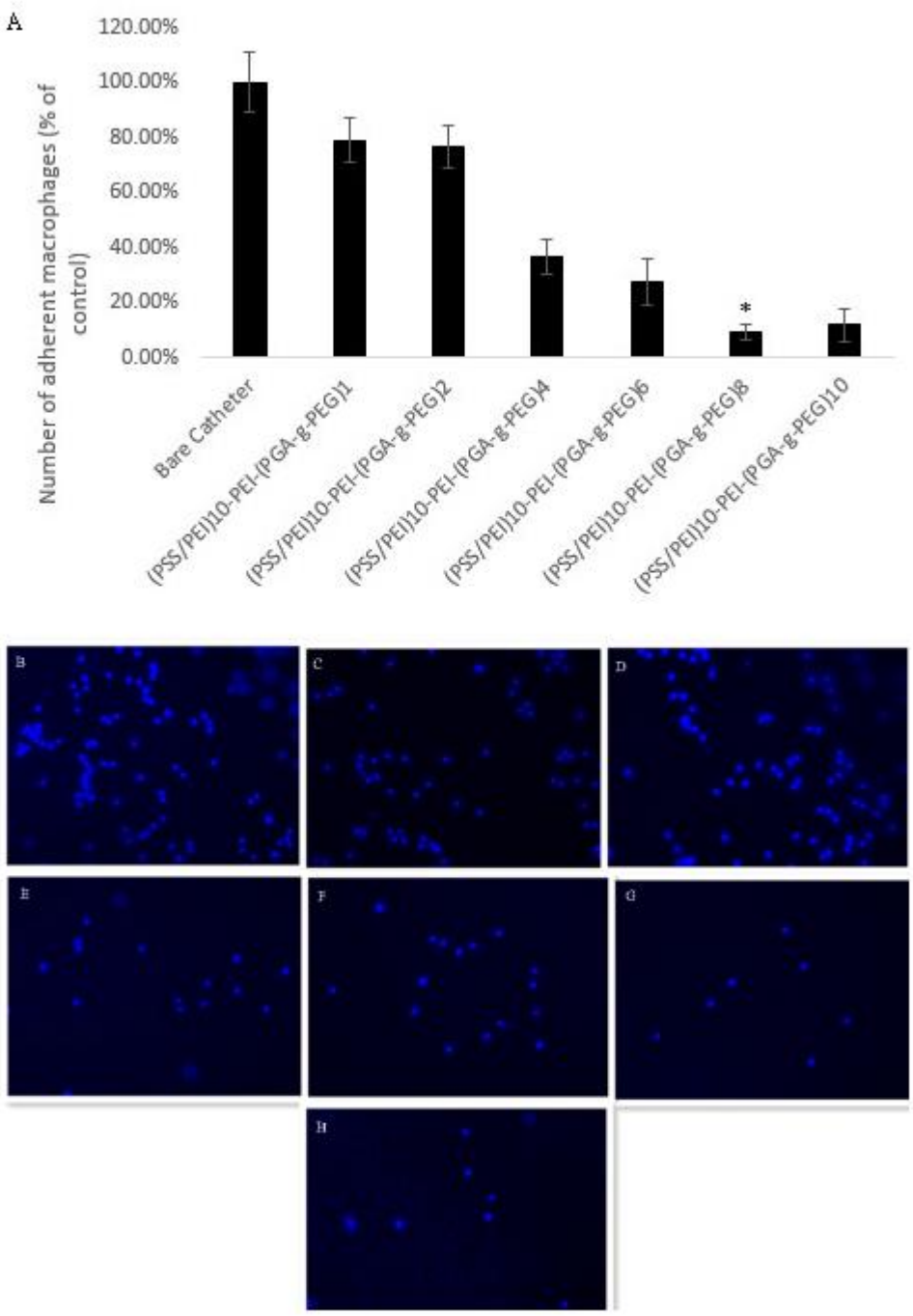

Fig. 11 Macrophage adhesion study. (A) DAPI fluorescent staining to quantify macrophage adhesion on bare catheters and $(PEI/PSS)_{10}$-PEI-(PGA-g-PEG/GA) coated catheters. *, $P<0.05$ compared to $(PEI/PSS)_{10}$-PEI-$(PGA\text{-}g\text{-}PEG/GA)_6$. Data shown are average ±SD (n = 4). (B)-(H) Remaining mouse macrophage adhesion on bare catheters,

$(PEI/PSS)_{10}$-PEI-$(PGA\text{-}g\text{-}PEG/GA)_1$, $(PEI/PSS)_{10}$-PEI-$(PGA\text{-}g\text{-}PEG/GA)_2$, $(PEI/PSS)_{10}$-PEI-$(PGA\text{-}g\text{-}PEG/GA)_4$, $(PEI/PSS)_{10}$-PEI-$(PGA\text{-}g\text{-}PEG/GA)_6$, $(PEI/PSS)_{10}$-PEI-$(PGA\text{-}g\text{-}PEG/GA)_8$, $(PEI/PSS)_{10}$-PEI-$(PGA\text{-}g\text{-}PEG/GA)_{10}$ after 24h, 37℃ culture.

Fig. 11 shows continuous reduction of macrophage adhesion when increasing the number of (PGA-g-PEG/GA) bilayers. With 1, 2, 4, 6, and 8 anti-adhesive bilayers, the number of macrophages was 79.1 ±8.1%, 76.7 ±7.6%, 36.8 ±8.5%, 27.2 ±8.5% and 9.2 ±4.5% of uncoated control. Further increasing the number of bilayers to 10 did not significantly charged the number of adhered macrophages. Thus, 8 bilayers of PGA-g-PEG/GA is sufficient to effectively inhibit macrophage adhesion.

PEG has a strong affinity for water molecules[37]. When PEG molecules are grafted on the surface of catheter, a layer with hydrated polymer will be formed, preventing cell adhesion. Thus, our anti-adhesive coating can potentially inhibit activated immune cells to assemble at the implant surface, and thereby prevent inflammatory response.

CONCLUSIONS

In this study we developed a novel antibacterial and anti-inflammatory coating for urinary catheters that is capable of sustained release of MH in urine for 33 days. MH is an effective antibiotic and anti-inflammatory drug. The coating has been shown to effectively inhibit biofilm formation by three bacterial species that are relevant to urinary tract infection, including multi-drug resistant *A. baumannii*. We also developed an anti-adhesive coating that can effectively inhibit adhesion of protein, bacteria, and macrophages. Moreover, all the coating materials are biocompatible polymers that are safe for human use. This work provides a reliable and promising approach for protecting implanted urinary catheters from infection and inflammation.

FUTURE DIRECTIONS

We have developed two separate LbL coatings: DS/MH/GA/AS/GC)$_{20}$, aiming at sustained release of MH to inhibit infection after urinary catheter insertion and long-term use, as well as (PGA-g-PEG/GA)$_8$, aiming at inhibit bacterial and cellular adhesion. The two coatings can be combined together to create a more effective antibacterial and anti-inflammatory coating. Another important aspect for this research is that the substrate we used in this study is PVC catheter. However, silicone catheter is more commonly used clinically. In the future, similar study needs to be conducted to create a suitable base layer for the silicone catheters, then the combined coating can be applied to silicone catheter to test its antibacterial and anti-inflammatory efficacy.

LIST OF REFERENCES

1. Maki, D.G. and P.A. Tambyah, *Engineering out the risk for infection with urinary catheters.* Emerging infectious diseases, 2001. 7(2): p. 342.

2. Garibaldi, R.A., et al., *An evaluation of daily bacteriologic monitoring to identify preventable episodes of catheter-associated urinary tract infection.* Infection Control, 1982: p. 466-470.

3. Karchmer, T.B., et al., *A randomized crossover study of silver-coated urinary catheters in hospitalized patients.* Archives of Internal medicine, 2000. 160(21): p. 3294.

4. Maki, D.G., C.H. Hennekens, and J.V. Bennett, *Prevention of catheter-associated urinary tract infection.* JAMA: the journal of the American Medical Association, 1972. 221(11): p. 1270-1271.

5. Ai, H., et al., *Coating and selective deposition of nanofilm on silicone rubber for cell adhesion and growth.* Cell biochemistry and biophysics, 2003. 38(2): p. 103-114.

6. Saint, S., et al., *The efficacy of silver alloy-coated urinary catheters in preventing urinary tract infection: a meta-analysis.* The American journal of medicine, 1998. 105(3): p. 236-241.

7. Gabriel, M.M., et al., *In vitro evaluation of the efficacy of a silver-coated catheter.* Current microbiology, 1996. 33(1): p. 1-5.

8. Tambyah, P.A., *Catheter-associated urinary tract infections: diagnosis and prophylaxis.* International journal of antimicrobial agents, 2004. 24: p. 44-48.

9. Darouiche Rabih O, et al., *Efficacy of antimicrobial-impregnated bladder catheters in reducing catheter-associated bacteriuria: a prospective, randomized, multicenter clinical trial.* Urology, 1999. 54(6): p. 976-981.

10. Venkataraman, P., C. Paramasivan, and R. Prabhakar, *In vitro activity of rifampicin, rifapentine and rifabutin against South Indian isolates of M. tuberculosis.* Indian Journal of Tuberculosis, 1993. 40(1): p. 17-20.

11. Anderson, J.M., *Biological responses to materials.* Annual Review of Materials Research, 2001. 31(1): p. 81-110.

12. Rolfe, B., et al., *The Fibrotic Response to Implanted Biomaterials: Implications for Tissue Engineering.* Regenerative Medicine and Tissue Engineering-Cells and Biomaterials. InTech, 2011: p. 551-68.

13. Cravens, D.D. and S. Zweig, *Urinary catheter management.* American family physician, 2000. 61(2): p. 369-376.

14. Hezi-Yamit, A., et al., *Impact of polymer hydrophilicity on biocompatibility: implication for DES polymer design.* Journal of Biomedical Materials Research Part A, 2009. 90(1): p. 133-141.

15. Lee, S.-D., et al., *Plasma-induced grafted polymerization of acrylic acid and subsequent grafting of collagen onto polymer film as biomaterials.* Biomaterials, 1996. 17(16): p. 1599-1608.

16. Okada, T. and Y. Ikada, *Surface modification of silicone for percutaneous implantation.* Journal of Biomaterials Science, Polymer Edition, 1996. 7(2): p. 171-180.

17. Lvov, Y., G. Decher, and H. Moehwald, *Assembly, structural characterization, and thermal behavior of layer-by-layer deposited ultrathin films of poly (vinyl sulfate) and poly (allylamine).* Langmuir, 1993. 9(2): p. 481-486.

18. Keller, S.W., H.-N. Kim, and T.E. Mallouk, *Layer-by-Layer Assembly of intercalation compounds and heterostructures on surfaces: Toward molecular" beaker" epitaxy.* Journal of the American Chemical Society, 1994. 116(19): p. 8817-8818.

19. Mendelsohn, J., et al., *Fabrication of microporous thin films from polyelectrolyte multilayers.* Langmuir, 2000. 16(11): p. 5017-5023.

20. Nieman, G. and B. Zerler, *A role for the anti-inflammatory properties of tetracyclines in the prevention of acute lung injury.* Current medicinal chemistry, 2001. 8(3): p. 317-325.

21. Soliman, G.M., et al., *Minocycline Block Copolymer Micelles and their Anti - Inflammatory Effects on Microglia.* Macromolecular bioscience, 2010. 10(3): p. 278-288.

22. Reis, C.P., et al., *Nanoparticulate biopolymers deliver insulin orally eliciting pharmacological response.* Journal of pharmaceutical sciences, 2008. 97(12): p. 5290-5305.

23. Lee, H., et al., *Mussel-inspired surface chemistry for multifunctional coatings.* Science, 2007. 318(5849): p. 426-430.

24. A, D.M., *Interrelationships between protein surface adsorption and bacterial adhesion.* Biotechnology and Genetic Engineering Reviews, 1998. 15: p. 413-437.

25. Harris, C.A., et al., *Reduction of protein adsorption and macrophage and astrocyte adhesion on ventricular catheters by polyethylene glycol and N-acetyl-L-cysteine.* J Biomed Mater Res A, 2011. 98(3): p. 425-33.

26. Park, K.D., et al., *Bacterial adhesion on PEG modified polyurethane surfaces.* Biomaterials, 1998. 19(7-9): p. 851-859.

27. Poncin-Epaillard, F. and G. Legeay, *Surface engineering of biomaterials with plasma techniques.* Journal of Biomaterials Science, Polymer Edition, 2003. 14(10): p. 1005-1028.

28. Morais, J.M., F. Papadimitrakopoulos, and D.J. Burgess, *Biomaterials/tissue interactions: possible solutions to overcome foreign body response.* The AAPS journal, 2010. 12(2): p. 188-196.

29. Boulmedais, F., et al., *Polyelectrolyte multilayer films with pegylated polypeptides as a new type of anti-microbial protection for biomaterials.* Biomaterials, 2004. 25(11): p. 2003-2011.

30. Fischer, D., et al., *In vitro cytotoxicity testing of polycations: influence of polymer structure on cell viability and hemolysis.* Biomaterials, 2003. 24(7): p. 1121-1131.

31. Anderson, G.G., et al., *Intracellular bacterial biofilm-like pods in urinary tract infections.* Science, 2003. 301(5629): p. 105-107.

32. Yang, L., et al., *Photocatalyzed surface modification of poly (dimethylsiloxane) with polysaccharides and assay of their protein adsorption and cytocompatibility.* Analytical chemistry, 2010. 82(15): p. 6430-6439.

33. Ratner, B., et al., *Biomaterials science: an introduction to materials in medicine.* San Diego, California, 2004: p. 162-164.

34. Roberts, J., E. Fussell, and M. Kaack, *Bacterial adherence to urethral catheters.* The Journal of urology, 1990. 144(2 Pt 1): p. 264.

35. Ong, Y.-L., et al., *Adhesion Forces between E. c oli Bacteria and Biomaterial Surfaces.* Langmuir, 1999. 15(8): p. 2719-2725.

36. Razatos, A., et al., *Force measurements between bacteria and poly (ethylene glycol)-coated surfaces.* Langmuir, 2000. 16(24): p. 9155-9158.

37. Harder, P., et al., *Molecular conformation in oligo (ethylene glycol)-terminated self-assembled monolayers on gold and silver surfaces determines their ability to resist protein adsorption.* The Journal of Physical Chemistry B, 1998. 102(2): p. 426-436.