In vitro evaluation of novel surface modifying molecules to prevent bacterial adhesion to urological devices

MP71-09

Christopher Munday[1,2,3,4], Alexandra Piotrowicz[5], Kyle MacDonald[1,2,5], Antonio Cillero[5], Jeannette Ho[5], John Denstedt[1,3], Jeremy P. Burton[1,2,3,4]

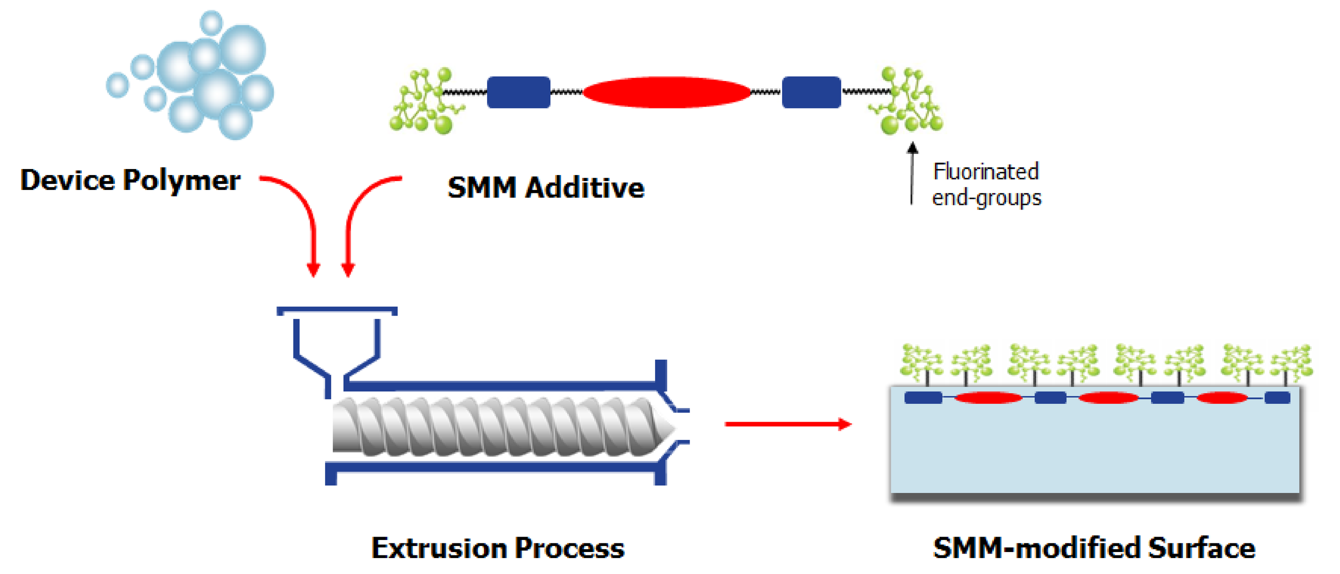
[1]Lawson Health Research Institute,[2]Canadian Centre for Human Microbiome and Probiotics [3]Division of Urology, Department of Surgery, Schulich School of Medicine [4]Department of Microbiology & Immunology, University of Western Ontario, London, Canada [5]Interface Biologics Inc., Toronto, Canada

Background and Objectives

- Bacterial adhesion and subsequent biofilm formation on urological devices can lead to device-associated urinary tract infections (UTIs) and other co-morbidities, thereby negatively impacting patient care and health economics.
- Catheter-associated UTIs are one of the most common healthcare infections and affect ~ 450,000 people in the USA annually.[1] The risk of infection increases 3 – 8 % per day of catheter placement, and all patients develop bacteriuria after one month.[2] Infections associated with stents occur in ~40% of cases.[2]
- Various methods of device modification have been investigated to reduce UTI rates, including incorporation of active antimicrobials that induce bacterial kill, as well as passive surface modification aimed at reducing bacterial adhesion to the device surface. However, successful implementation of these modifications in commercial products and their clinical efficacy have been limited.
- Eluting antimicrobials can lose potency over time as the active compound becomes depleted, loses biological activity, or its diffusion is hampered due to biofouling on the device surface. Eluting antibiotics are of additional concern due to the potential for development of antibiotic resistance.
- The objective of this study was to investigate the ability of a passive surface modification technology utilizing fluorinated surface modifying macromolecules (SMMs), which can be incorporated into a device during the manufacturing process, to reduce bacterial adhesion and biofilm formation on prototype surfaces. SMMs are used clinically in intravascular devices to resist platelet adhesion and thrombus formation.[3]

Methods – Surface Modification

- Unmodified Control and SMM-modified polyurethane rod or urological tubing prototypes were prepared using standard melt extrusion processes at 200 – 230°C.
- SMMs were compounded into the polyurethane resin (Carbothane 85A) at a loading of 2% (by weight) and migrated to the prototype surface during extrusion.
- Surface modification was confirmed by X-ray photoelectron spectroscopy (~25 atomic % fluorine detected on SMM prototypes). Both inner and outer surfaces of tubing were simultaneously modified.

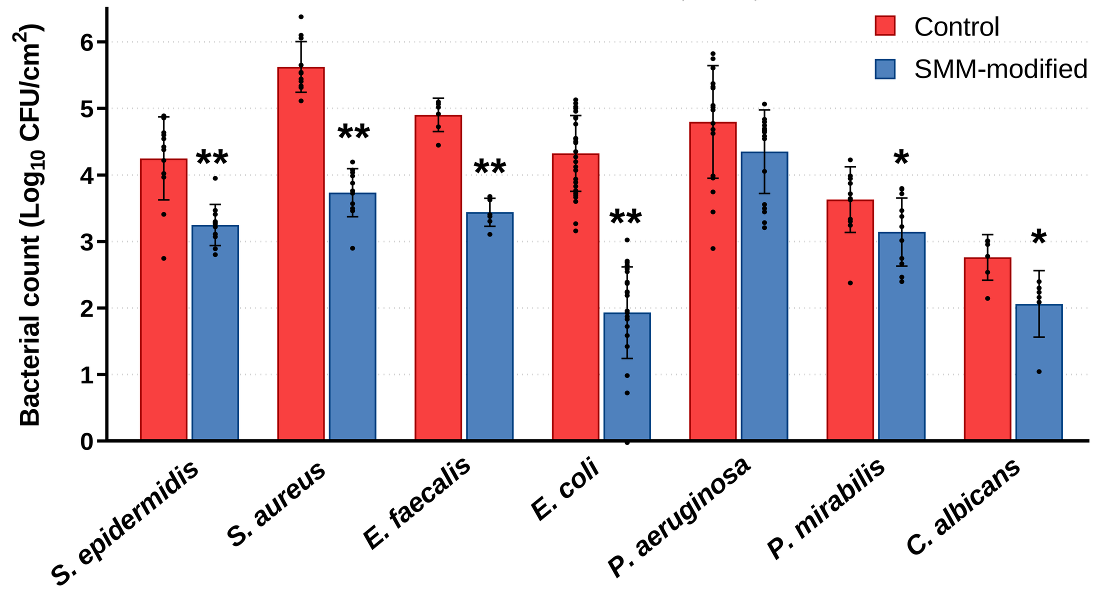


Methods – Bacterial Adhesion Experiments

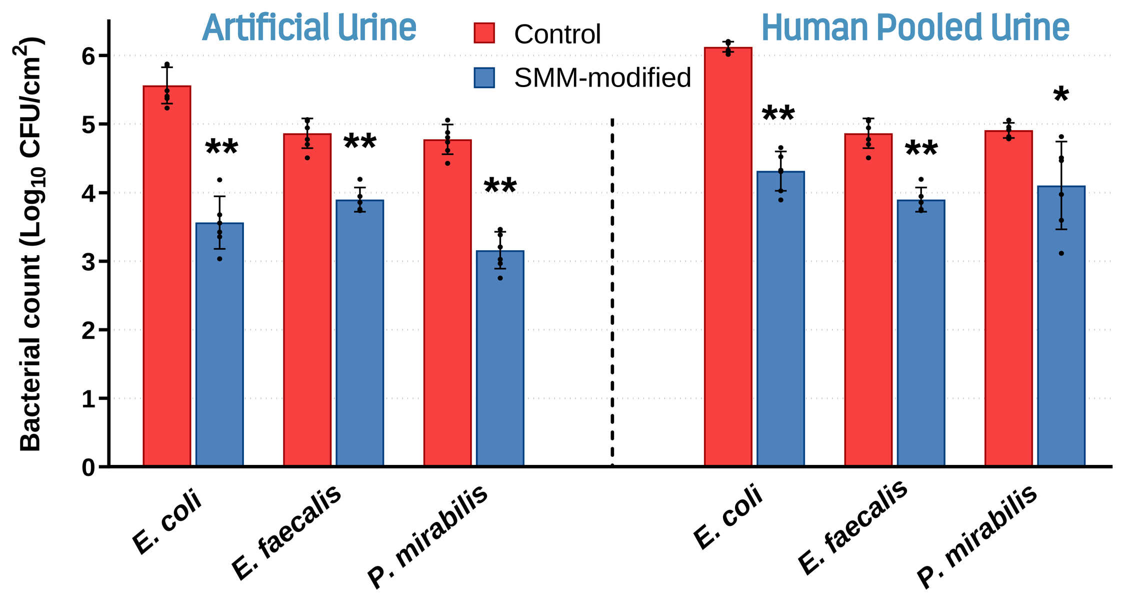
- Static conditions: Rod samples (1.5 cm long) were incubated for 2h at 37°C in PBS, artificial urine, or human pooled urine (3 donors) inoculated with 10^8 cfu/ml bacteria or fungi. After incubation, samples were rinsed with PBS and sonicated for 30 min to detach adhered bacteria. Sonicated solutions were diluted and spot plated to enumerate bacteria via colony counts.
- Flow conditions: A peristaltic pump was used to pump artificial urine inoculated with 10^8 cfu/ml *E.coli* through test tubing at 0.5 ml/min and 37°C for 2h to seed bacteria on the luminal surface, followed by sterile artificial urine flow for 3 days. Samples (1.5 cm long) were cut from the circuit and processed for colony counts as described above. Samples were also stained using crystal violet to visualize biofilm formation and dehydrated, dried and imaged by SEM.
- Bacterial adhesion from clinical urine was evaluated by incubating tubing samples for 24h at 37°C in urine collected from patients with indwelling ureteral stents.
- Statistically significant differences in adhesion were assessed using student's t-test (*$p < 0.05$; **$p < 0.005$). Error bars represent standard deviations of the mean.

Results – Static Experiments

Microbial adhesion to polyurethane rods after 2h incubation in inoculated PBS (n > 6)

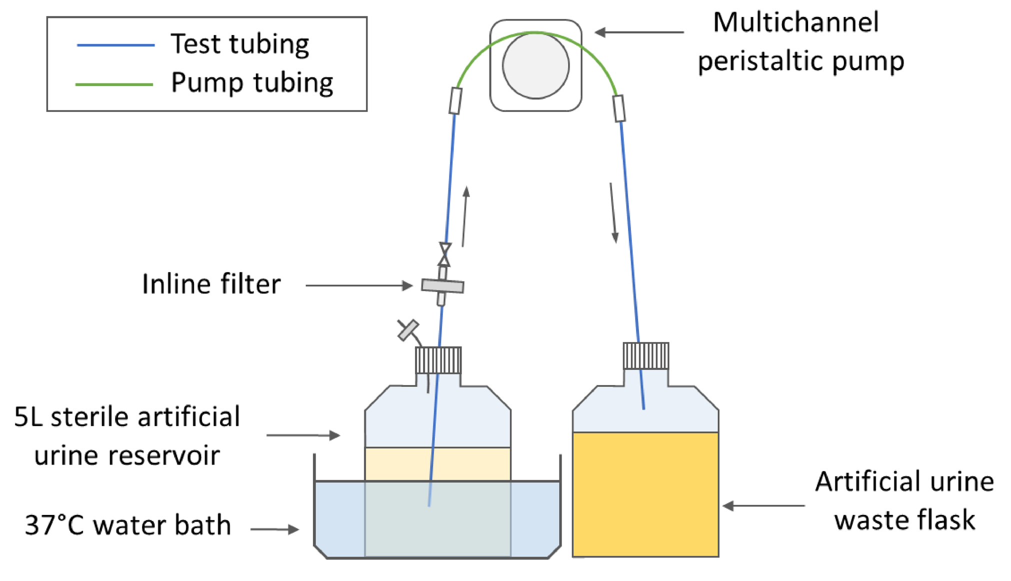


Uropathogen adhesion to polyurethane rods after 2h incubation in Artificial or Human Pooled Urine (n = 6)

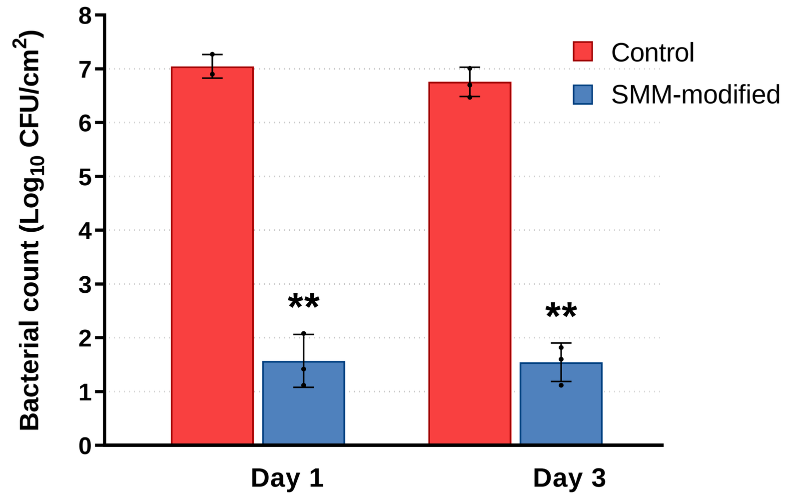


Results – Flow Experiments

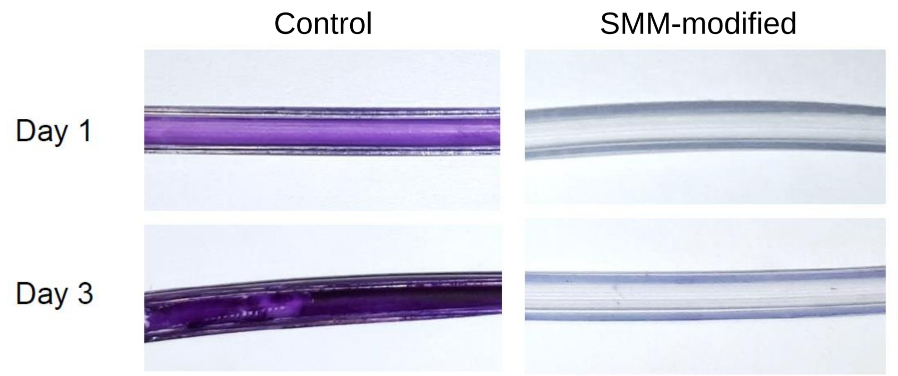
Flow System set-up for evaluating bacterial adhesion under flow conditions



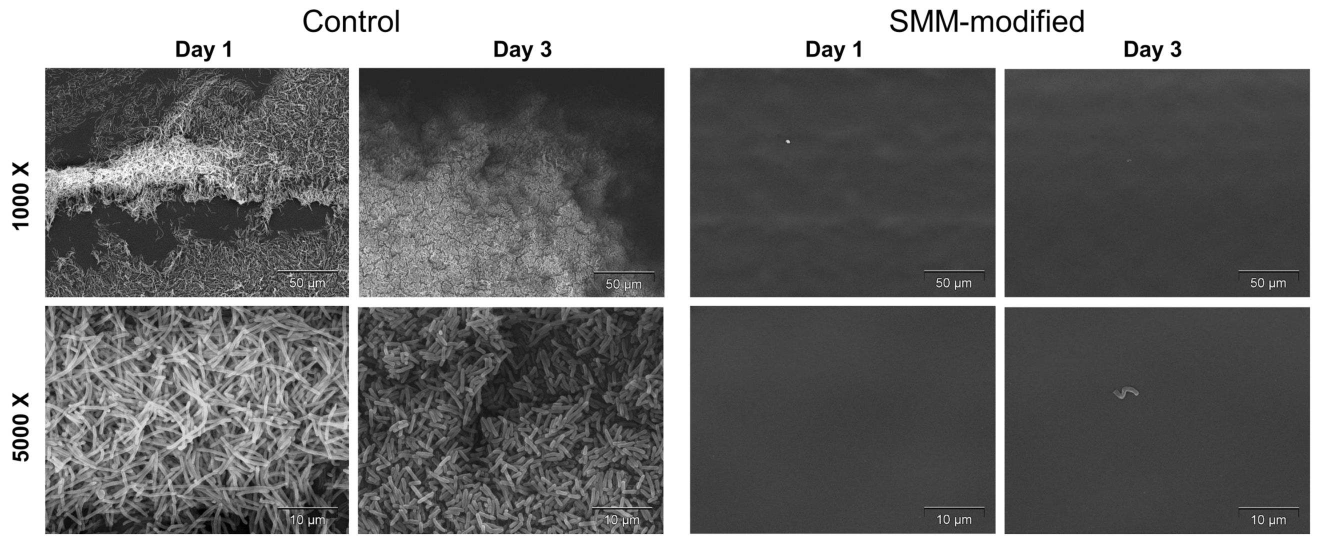
***E. coli* adhesion to polyurethane tubing under flow conditions (n = 3)**



Crystal violet staining of biofilm on tubing intraluminal surfaces

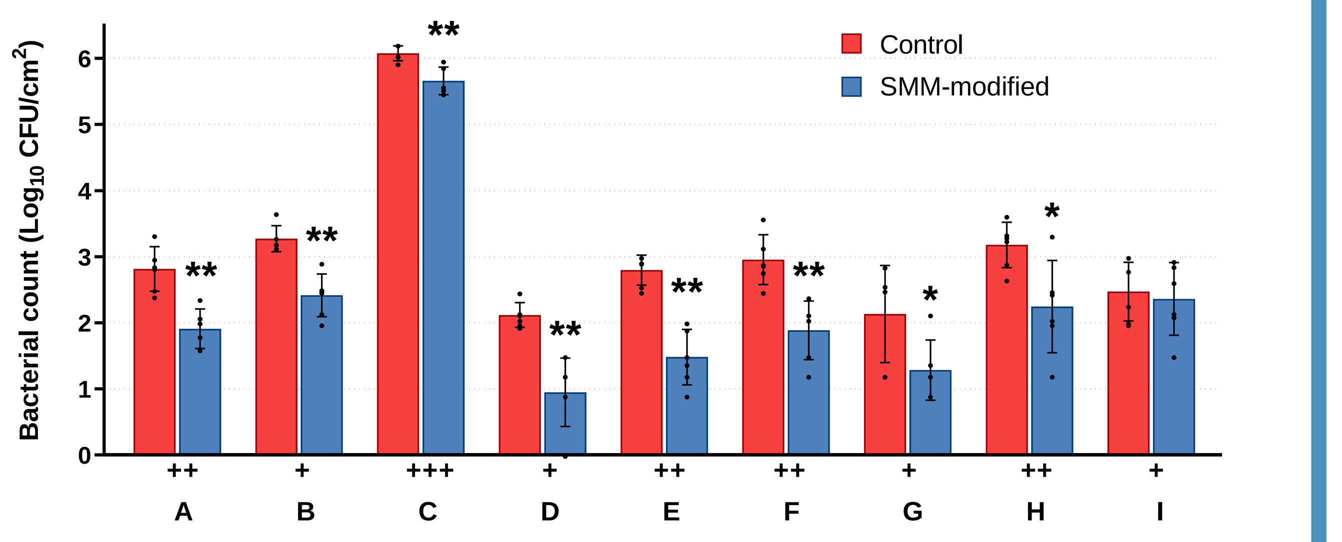


Scanning electron microscopy images of biofilm on tubing intraluminal surfaces



Results – Clinical Urine Experiments

Overall bacterial adhesion to polyurethane tubing after 24 h incubation in clinical urine specimens obtained from patients with ureteral stents (n > 6)



\+ Represents the number of major colony types cultured from the sample

A - I Represent individual clinical urine specimens

Conclusions and Outlook

- SMM modification of polyurethane prototypes resulted in a reduction in bacterial adhesion of up to 2 log (or 99%) when tested with a variety of microbial species (gram positive bacteria, gram negative bacteria and yeast), suggesting an overall anti-adhesive property of the modified surfaces.
- Reductions in bacterial adhesion were confirmed with 3 uropathogens in artificial and human pooled urine. Furthermore, when incubated in clinical urine specimens containing mixed bacterial populations, SMM-modified samples reduced attachment compared to unmodified controls in 8 out of 9 cases.
- Experiments under flow conditions showed potential of the SMM-modified surfaces to resist bacterial adhesion in dynamic environments and over extended time frames, with 5 log lower *E. coli* attachment to modified tubing vs control tubing at both 1 and 3 days post inoculation. Corresponding reductions in biofilm formation were visible through crystal violet staining and SEM imaging.
- SMM modification of urological devices may be an effective strategy to reduce bacterial adhesion and biofilm formation, which has the potential to reduce device-related UTIs and make a meaningful positive impact on patient outcomes.

Acknowledgments

References:

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2. Ramstedt, M. et al. Evaluating Efficacy of Antimicrobial and Antifouling Materials for Urinary Tract Medical Devices: Challenges and Recommendations. *Macromolecular Bioscience* 2019, e1800384 .
3. McDiarmid, S et al. Outcomes in a nurse-led peripherally inserted central catheter program: a retrospective cohort study. *CMAJ Open* 2017, Vol 5(3):E535-539.

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