Development of Dual Anti-biofilm and Anti-bacterial Medical Devices

Electronic Supplementary Information

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Figure S1. NMR spectrum for p-EGDPEA.
Figure SI2. NMR spectrum for p-DEGMA.
Figure SI3. NMR spectrum for p-HPhOPA.

Figure SI4. Zone of inhibition measured for *S. aureus* (■) and *E. coli* (■) for various polymers with varied CLogP. Error bars equal ± one standard deviation unit, N = 3.

**Optimisation of Polymer Coating**

Optimisation of the p-tBCHA coating coating thickness was necessary due to the inability of this coating on an impregnated device to achieve a zone of inhibition for *E. coli*. Polymer coating was assessed in parallel as a control. Serial plate transfer tests using plates inoculated with *E. coli* were carried out on impregnated catheter sections dip-coated three times with 1 or 5 wt% polymer in toluene solutions. The zone of inhibition diameter for the two polymer solution concentrations was normalised relative to the uncoated impregnated catheter; dip-coating using the lower concentration polymer solution resulted in an increase in zone of inhibition size relative to the 5 wt% polymer solution (Figure SI5a). This is likely due to the low polymer concentration producing a thinner coating that only minimally inhibited antibiotic elution. The effect of polymer molecular weight on drug release was next investigated using catheter sections dip-coated with 1 wt% in toluene solutions of p-tBCHA and p-EGDPEA synthesised at various molecular weights (Figure SI6). The *E. coli* tK100 test was repeated using these catheter sections; both polymers showed that an optimal molecular weight of < 6.5 × 10⁴ g mol⁻¹ would permit sufficient antibiotic elution to successfully kill all attached bacteria at 72 hours (Figure SI5b). Tunable polymeric hydrogel systems for drug delivery have previously been reported, with polymer network and mesh size affecting drug-polymer interactions.² There was no difference in bacterial viability between the non-impregnated coated catheters and non-impregnated uncoated control (Figure SI5c), confirming that the anti-biofilm coatings were not antibacterial.
Figure S15. Optimisation of the delivery of anti-microbials. a) Normalised zone of inhibition diameter (for E. coli) relative to uncoated impregnated catheter for varied polymer solution concentrations (wt%). Scale bars equal ± one standard deviation, n = 3. b-c) tK100 with b) impregnated and c) plain catheters coated with p-tBCHA or pEGDPEA at various polymer molecular weights. Scale bars equal ± one standard deviation, n = 3.
Figure SI6. GPC traces for polymers a) p-tBCHA, b) p-EGDPEA.
Figure S17. LCMS spectra showing retention times (RT) and detected mass for standard solutions of a) triclosan RT: 3.67 min; [M-H]-
: C₁₄H₁₀Cl₃O₂ found 286.9440, requires 286.9433, b) sparfloxacin RT: 2.54 min; [M+H]+: C₁₉H₂₈F₂N₄O₃ found 393.1746, requires
393.1738 and c) rifampicin RT: 3.27 min; [M+H]+: C₄₃H₅₀N₁₂O₁₂ found 823.4139, requires 823.4129.
Figure SI8. LCMS spectra of the supernatant taken after incubation with a plain catheter. Ranges associated with peaks for a) triclosan, b) sparfloxacin and c) rifampicin assessed, as determined from standard solutions (Figure SI7).
Figure S19. LCMS spectra of the supernatant taken after incubation with an impregnated catheter coated with p-tBCHA. Ranges associated with peaks for a) triclosan RT: 3.70 min; [M-H]: C\textsubscript{12}H\textsubscript{2}Cl\textsubscript{2}O\textsubscript{2} found 286.9443, requires 286.9433, b) sparfloxacin RT: 2.50 min; [M+H]\textsuperscript{+}: C\textsubscript{19}H\textsubscript{2}F\textsubscript{3}N\textsubscript{3}O\textsubscript{3} found 393.1736, requires 393.1738 and c) rifampicin RT: 3.22 min; [M+H]\textsuperscript{+}: C\textsubscript{44}H\textsubscript{56}N\textsubscript{8}O\textsubscript{12} found 823.4153, requires 823.4129 assessed, as determined from standard solutions (Figure S17).
Figure S10. LCMS spectra of the supernatant taken after incubation with an impregnated catheter coated with p-EGDPEA. Ranges associated with peaks for **a)** triclosan RT: 3.69 min; [M-H]: C₁₂H₂₂Cl₂O₂ found 286.9443, requires 286.9433, **b)** spiramycin RT: 2.47 min; [M+H]: C₁₀H₂₂Br₂N₂O₃ found 393.1738, requires 393.1738 and **c)** rifampin RT: 3.21 min; [M+H]: C₄₄H₅₀NiO₁₂ found 823.4151, requires 823.4129 assessed, as determined from standard solutions (Figure S17).
Figure S11. LCMS spectra of the supernatant taken after incubation with an impregnated catheter coated with p-DEGMA. Ranges associated with peaks for a) triclosan RT: 3.68 min; [M-H]: C12H2ClO2 found 286.9442, requires 286.9433, b) sparfloxacin RT: 2.47 min; [M+H]: C13H23F3N4O3 found 393.1737, requires 393.1738 and c) rifampicin RT: 3.26 min; [M+H]: C44H69N9O12 found 823.4130, requires 823.4129 assessed, as determined from standard solutions (Figure S17).
Figure SI12. LCMS spectra of the supernatant taken after incubation with an impregnated catheter coated with p-HPhOPA. Ranges associated with peaks for **a**) triclosan RT: 3.68 min; [M-H]: C_{12}H_{2}Cl_{6}O_{2} found 286.9442, requires 286.9433, **b**) sparfloxacin RT: 2.48 min; [M+H]: C_{10}H_{3}F_{5}N_{3}O_{3} found 393.1738, requires 393.1738 and **c**) rifampicin RT: 3.23 min; [M+H]: C_{43}H_{50}N_{14}O_{22} found 823.4145, requires 823.4129 assessed, as determined from standard solutions (**Figure SI7**).
Figure S13. LCMS spectra of the supernatant taken after incubation with an uncoated impregnated catheter. Ranges associated with peaks for a) triclosan RT: 3.67 min; [M-H]: C₁₉H₂₃Cl₂O₂ found 286.9442, requires 286.9433, b) sparfloxacin RT: 2.47 min; [M+H]: C₂₀H₂₃F₂N₂O₂ found 393.1737, requires 393.1738 and c) rifampicin RT: 3.20 min; [M+H]: C₃₀H₅₀N₂O₁₂ found 823.4144, requires 823.4129 assessed, as determined from standard solutions (Figure S17).
Figure S114. Time-of-flight secondary ion mass spectrometry depth profile for a) p-tBCHA for ions C₈H₉⁺ ( ), C₆H₁₁⁺ ( ), SiC₃H₉⁺ ( ), Si₂C₆H₁₀O⁺ ( ), C₅H₁₁N₂⁺ ( ), C₁₀H₁₂N₂O₂F₂⁺ ( ), C₁₂H₂O₂Cl⁺ ( ), Si⁺ ( ), C₃H₈O⁺C₁₂⁺ ( ); and b) p-EGDPEA for ions C₅H₇⁺ ( ), C₇H₇⁺ ( ), C₅H₁₁N₂⁺ ( ), SiC₃H₉⁺ ( ), Si₂C₆H₁₀O⁺ ( ), C₁₀H₁₂N₂O₂F₂⁺ ( ), C₁₂H₂O₂Cl⁺ ( ), Si⁺ ( ), C₃H₈O⁺C₁₂⁺ ( ).
Figure S115. Time-of-flight secondary ion mass spectrometry depth profile for a) p-DEGMA for ions $C_3H_7O^+$, $C_2H_5O^+$, $SiC_3H_9^+$, $C_5H_{11}N_2^+$, $Si_2C_5H_{15}O^+$, $C_{15}H_22N_4O_3F_2^+$, $C_{19}H_{22}N_4O_3F_2^+$, $C_{12}H_7O_2Cl^+$, $C_{43}H_{58}N_4O_{12}^+$; and b) p-HPhOPA for ions $SiC_3H_9^+$, $Si_2C_5H_{15}O^+$, $C_{15}H_22N_4O_3F_2^+$, $C_{19}H_{22}N_4O_3F_2^+$, $C_{12}H_7O_2Cl^+$, $C_{43}H_{58}N_4O_{12}^+$. 
Figure S116. Time-of-flight secondary ion mass spectrometry depth profile for a) an uncoated impregnated catheter and b) a catheter as received for ions SiC_3H_9^+( ), SiC_3H_4O^+( ), Si^+( ), C_8H_11N_2^+( ), C_19H_22N_4O_3F_2^+( ), C_12H_7O_2Cl_3^+( ), C_83H_88N_4O_12^+( ).
Figure SI17. Determination of macrophage viability in response to uncoated or coated antibiotic-impregnated catheters in a) unstimulated and b) LPS-stimulated conditions.¹

Figure SI18. NMR spectrum for p-tBCHA.

References

¹ The high LDH signal observed for impregnated catheters is due, at least in part, to fluorescence associated with the antimicrobials. To gain a measure of the viability associated with the coatings, LDH measurements were undertaken on coated non-impregnated catheters.