Supporting Information

Precisely Structured Nitric-Oxide-Releasing Copolymer Brush Defeats Broad-Spectrum Catheter-Associated Biofilm Infections *In Vivo*

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Keywords: broad-spectrum antibiofilm; antithrombogenic; structured polymer; nitric oxide; surface hydrophilicity

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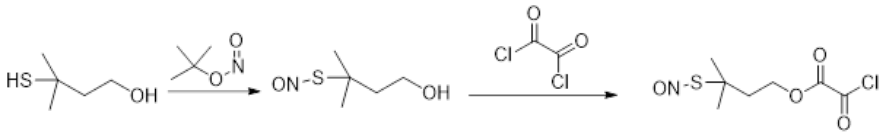


Scheme S1. Synthesis of NO release precursor (NTMB-Cl)

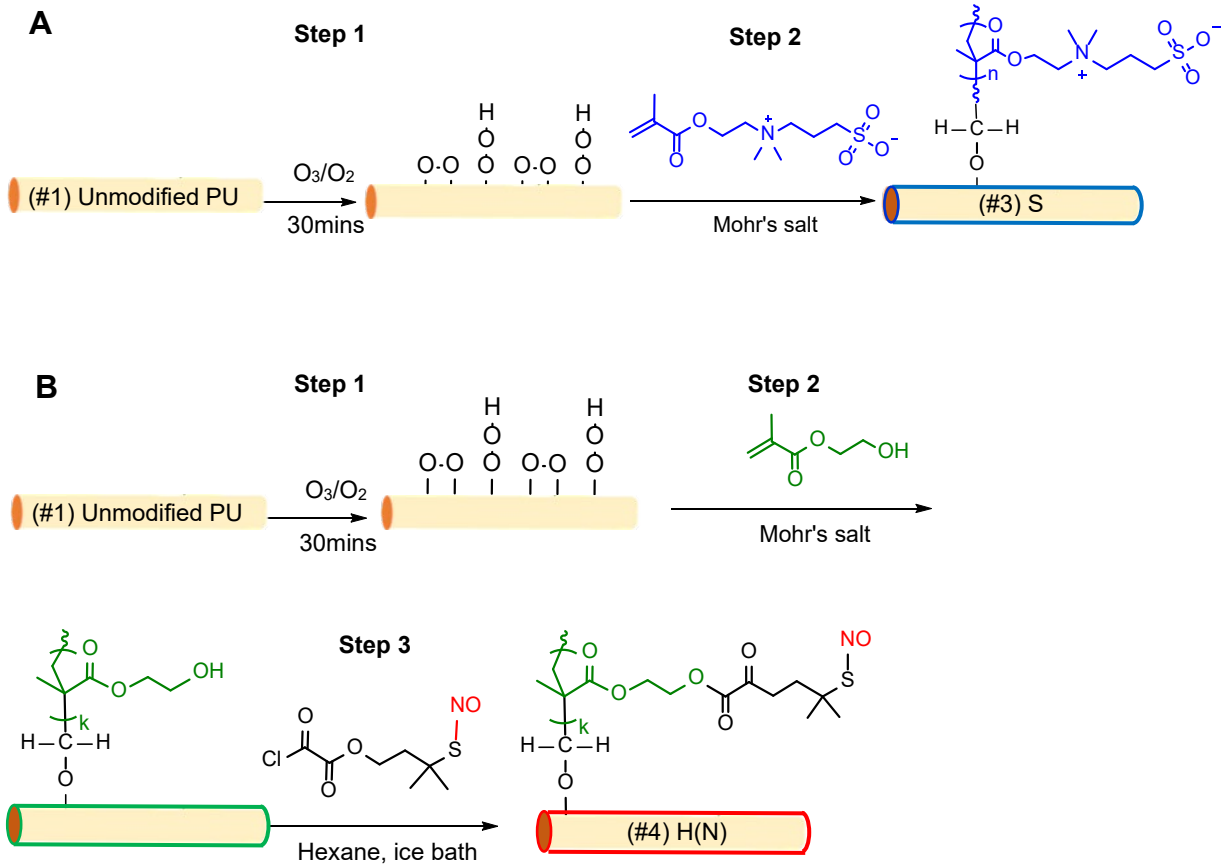


Figure S1. Synthesis of **(A)** homo poly(SBMA) coating ((#3) S) and **(B)** homo poly(HEMA-NO) coating ((#4) H(N)).

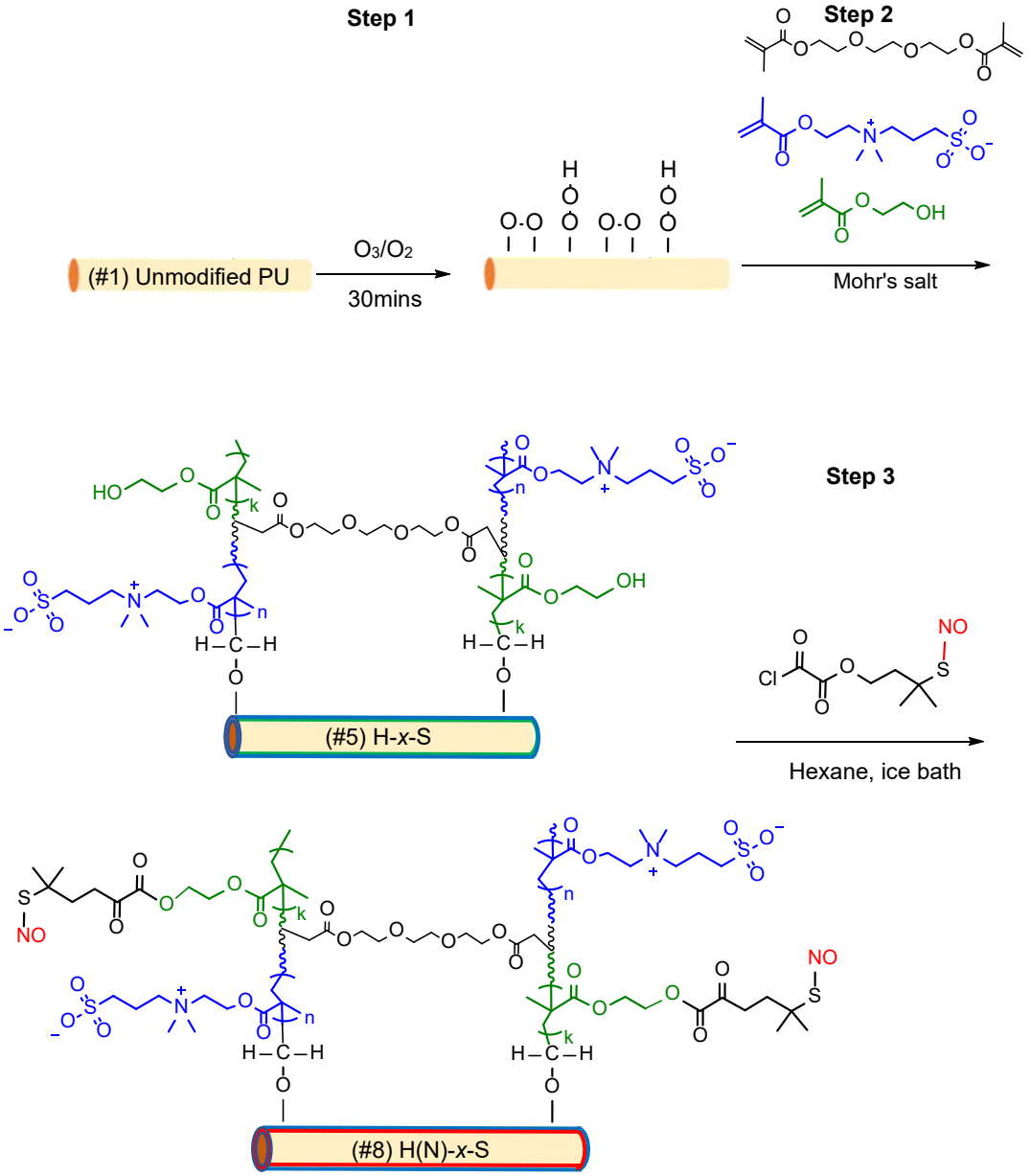


Figure S2. Synthesis of crosslinked coating ((#5) H-*x*-S and (#8) H(N)-*x*-S).

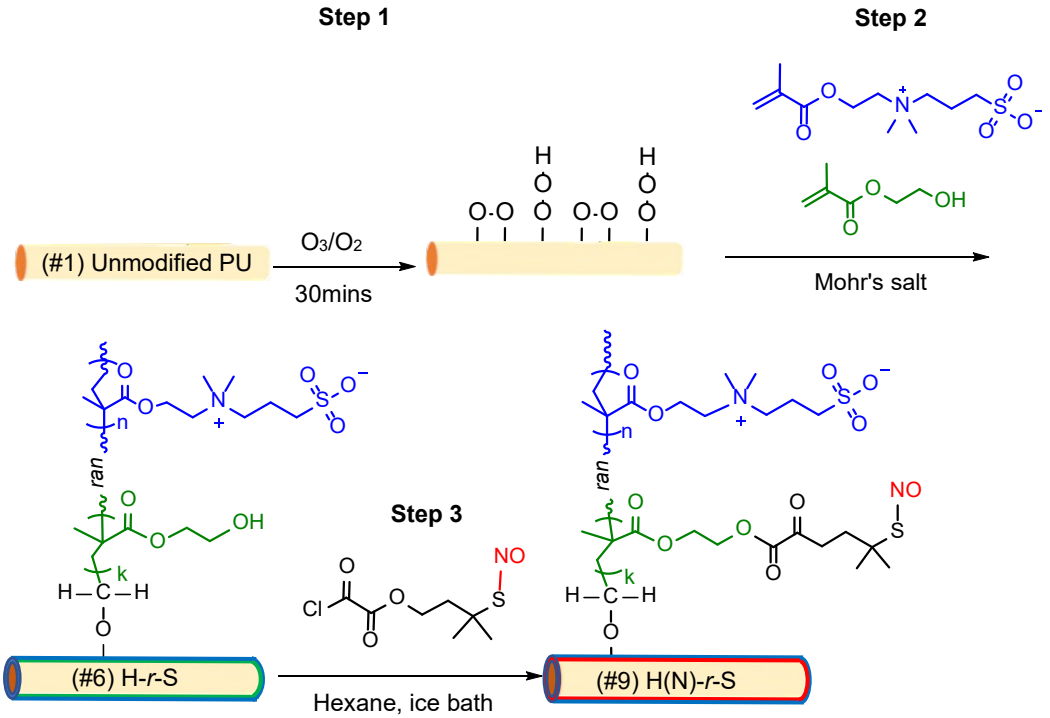


Figure S3. Synthesis of random copolymer coating ((#6) H-*r*-S and (#9) H(N)-*r*-S).

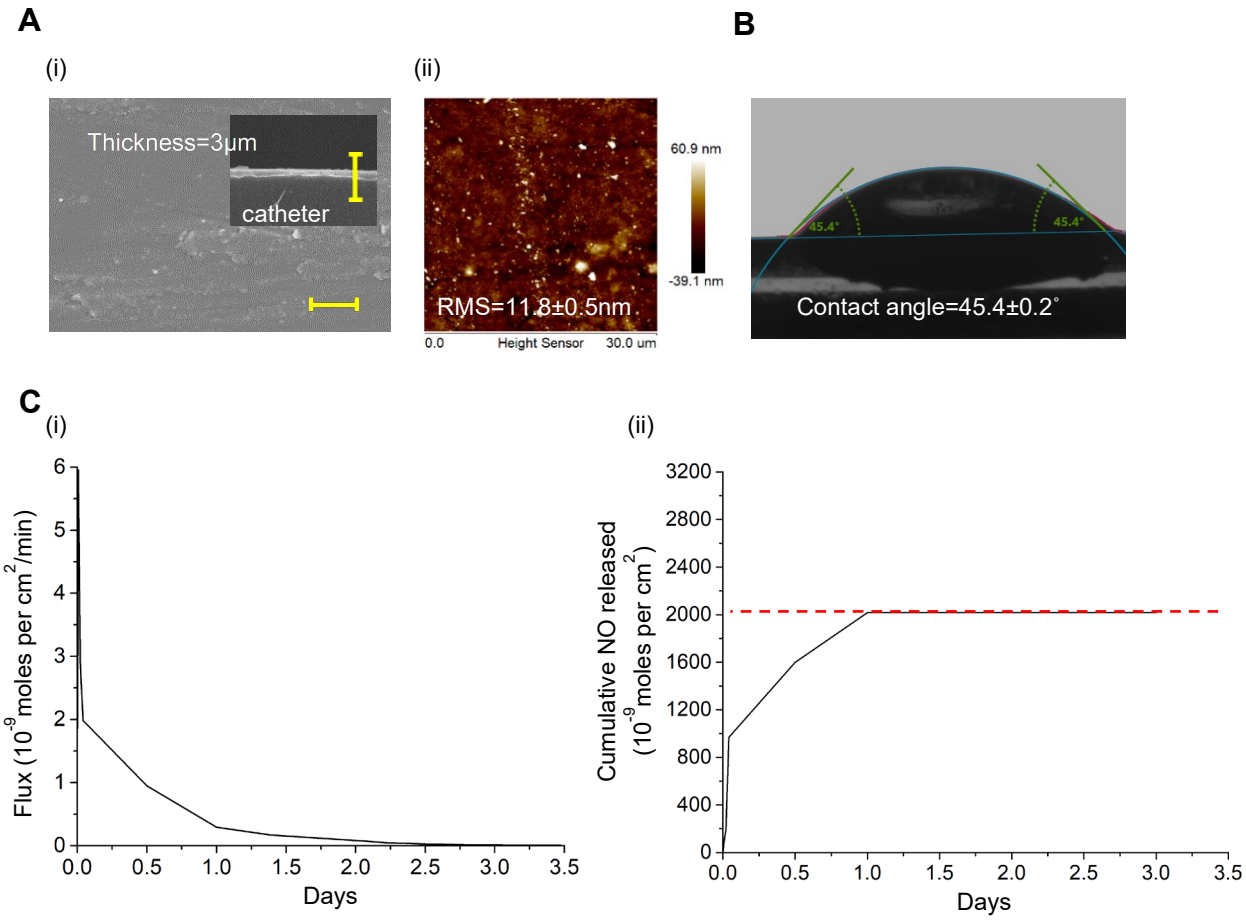


Figure S4. Further characterization of (#10) H(N)-*b*-S **(A)** characterization of poly(HEMA) which is the first step in the synthesis of (#10) H(N)-*b*-S: Surface morphology (i) SEM image of catheter surface and cross section (inset) (scale bar=10 µm), (ii) AFM image of surface morphology with measured root mean square height variation. **(B)** contact angle of poly(HEMA). **(C)** Quantification of NO-release precursors on (#10) H(N)-*b*-S: (i) NO flux profile at 55 ˚C, (ii) Cumulative NO released.

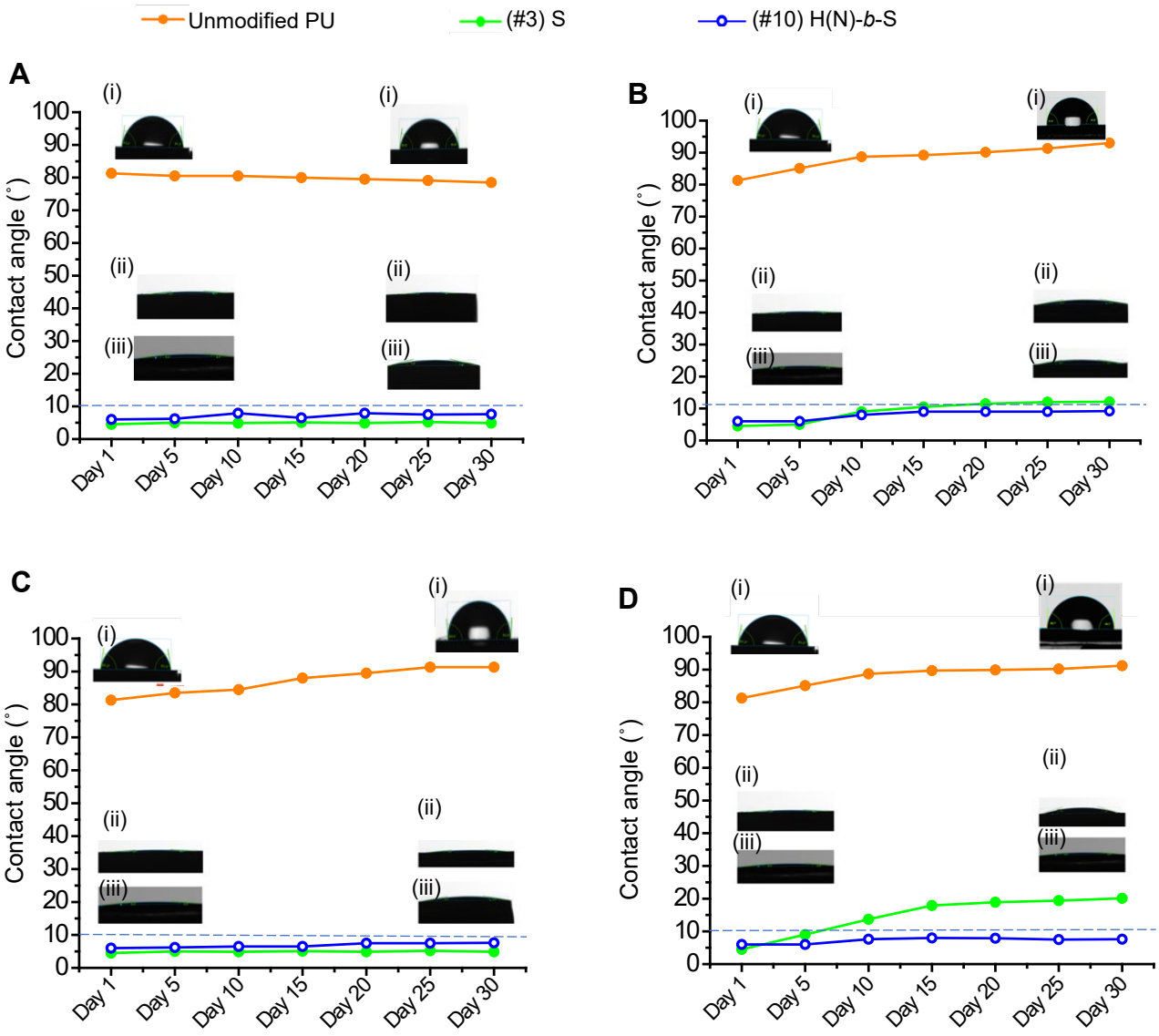


Figure S5. Stability test of coatings. Long-term hydrophilicity test under various conditions: **(A)** PBS saline **(B)** Serum **(C)** *S. aureus* inoculum (D) *P. aeruginosa* inoculum. The contact angles are shown in the insets ((i) (#1) unmodified PU, (ii) (#3) S, (iii) (#10) H(N)-*b*-S), the dotted line in the graphs indicates 10˚.

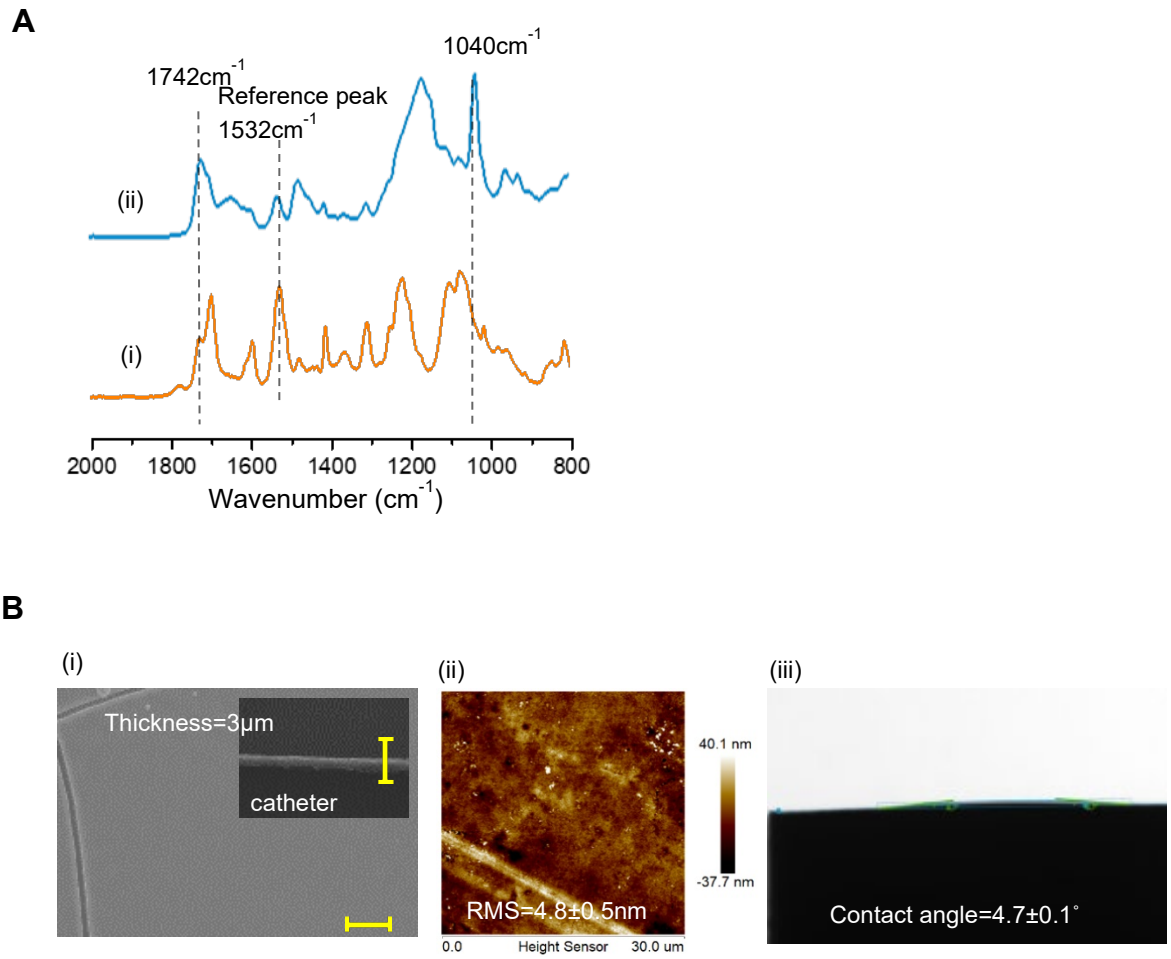


Figure S6. Characterization of coating (#3) S. **(A)** FTIR spectra of catheter samples: (i) unmodified control, (ii) (#3) S, characterization peaks: C=O ester at 1742 cm^{-1} and SO_3^- sulfonyl peak at 1040 cm^{-1}. **(B)** (i) SEM image of catheter surface and cross section (inset) (scale bar=10 µm), (ii) AFM image with measured root mean square height variation, (iii) contact angle.

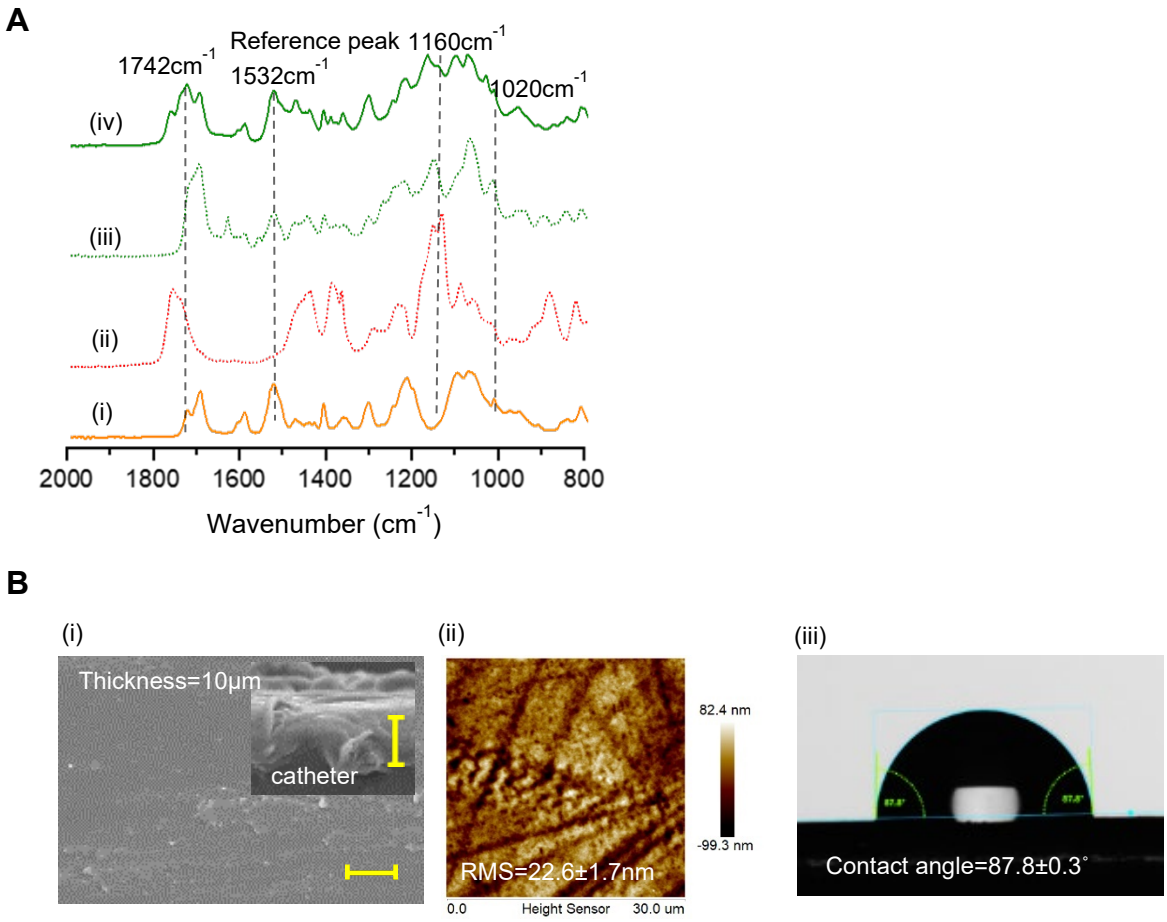


Figure S7. Characterization of coating (#4) H(N). **(A)** FTIR spectra of catheter samples: (i) unmodified control, (ii) NO-donor NTMB-Cl, (iii) homo poly(HEMA) coating, (iv) (#3) H(N) coating with characterization peaks: C=O ester at 1742 cm^{-1} and RSNO peak at 1160 cm^{-1}. **(B)** (i) SEM image of catheter surface and cross section (inset) (scale bar=10 µm), (ii) AFM image with measured root mean square height variation, (iii) contact angle.

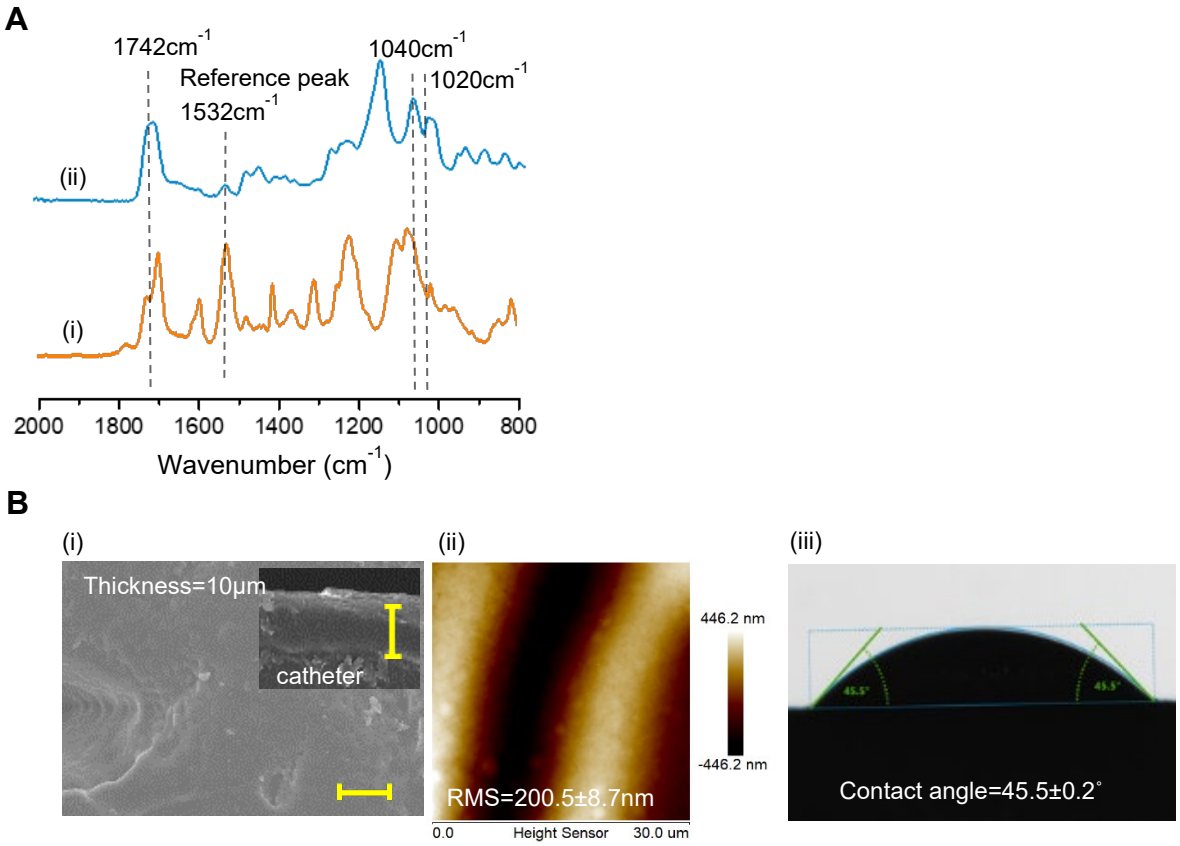


Figure S8. Characterization of coating (#5) H-*x*-S. **(A)** FTIR spectra of catheter samples: (i) unmodified control, (ii) (#5) H-*x*-S with characterization peaks: C=O ester at 1742 cm^{-1}, SO_3^- sulfonyl peak at 1040 cm^{-1} and C-O-H peak at 1020 cm^{-1}. **(B)** (i) SEM image of catheter surface and cross section (inset) (scale bar=10 μm), (ii) AFM image with measured root mean square height variation, (iii) contact angle.

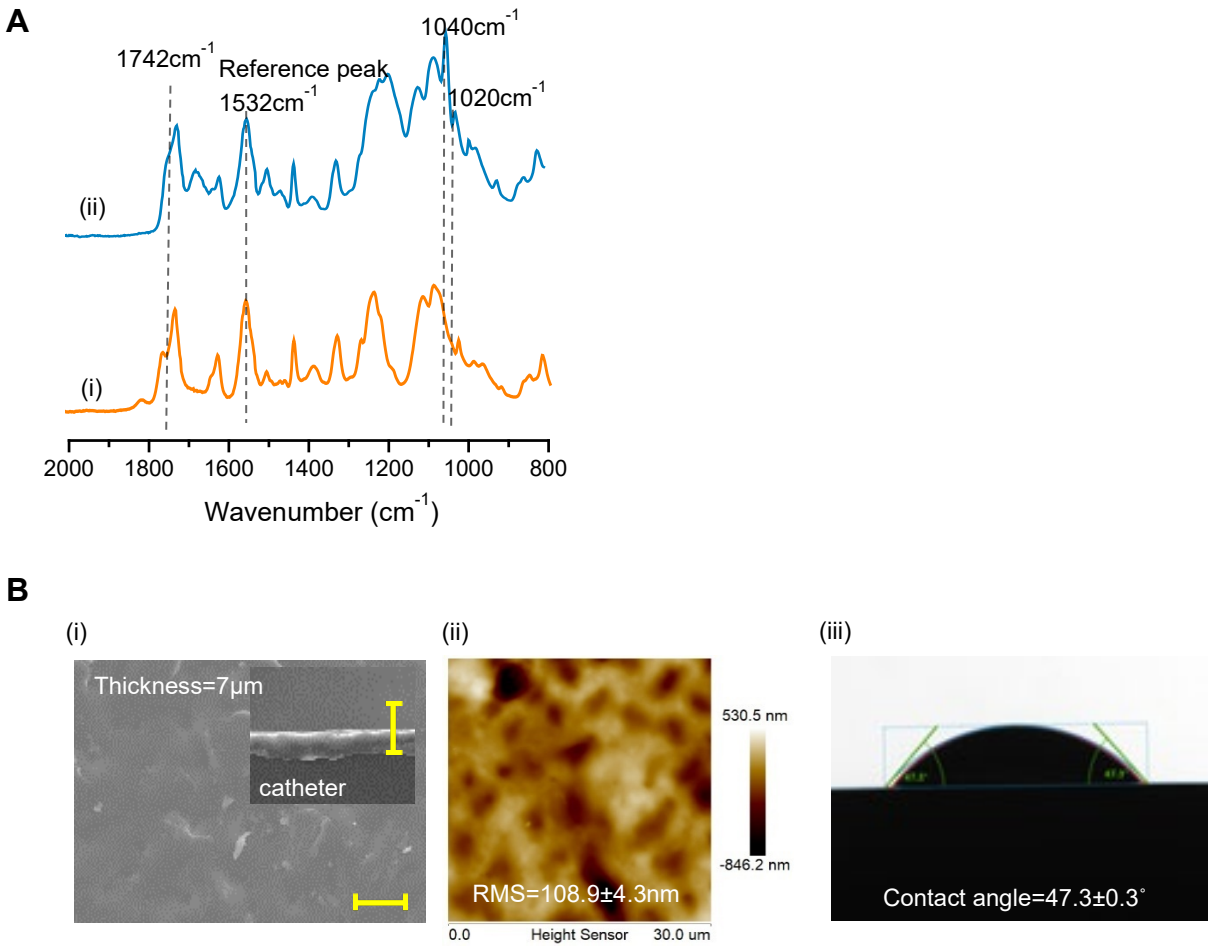


Figure S9. Characterization of coating (#6) H-*r*-S. **(A)** FTIR spectra of catheter samples: (i) unmodified control, (ii) (#6) H-*r*-S with characterization peaks: C=O ester at 1742 cm^{-1}, SO_3^- sulfonyl peak at 1040 cm^{-1} and C-O-H peak at 1020 cm^{-1}. **(B)** (i) SEM image of catheter surface and cross section (inset) (scale bar=10 µm), (ii) AFM image with measured root mean square height variation, (iii) contact angle.

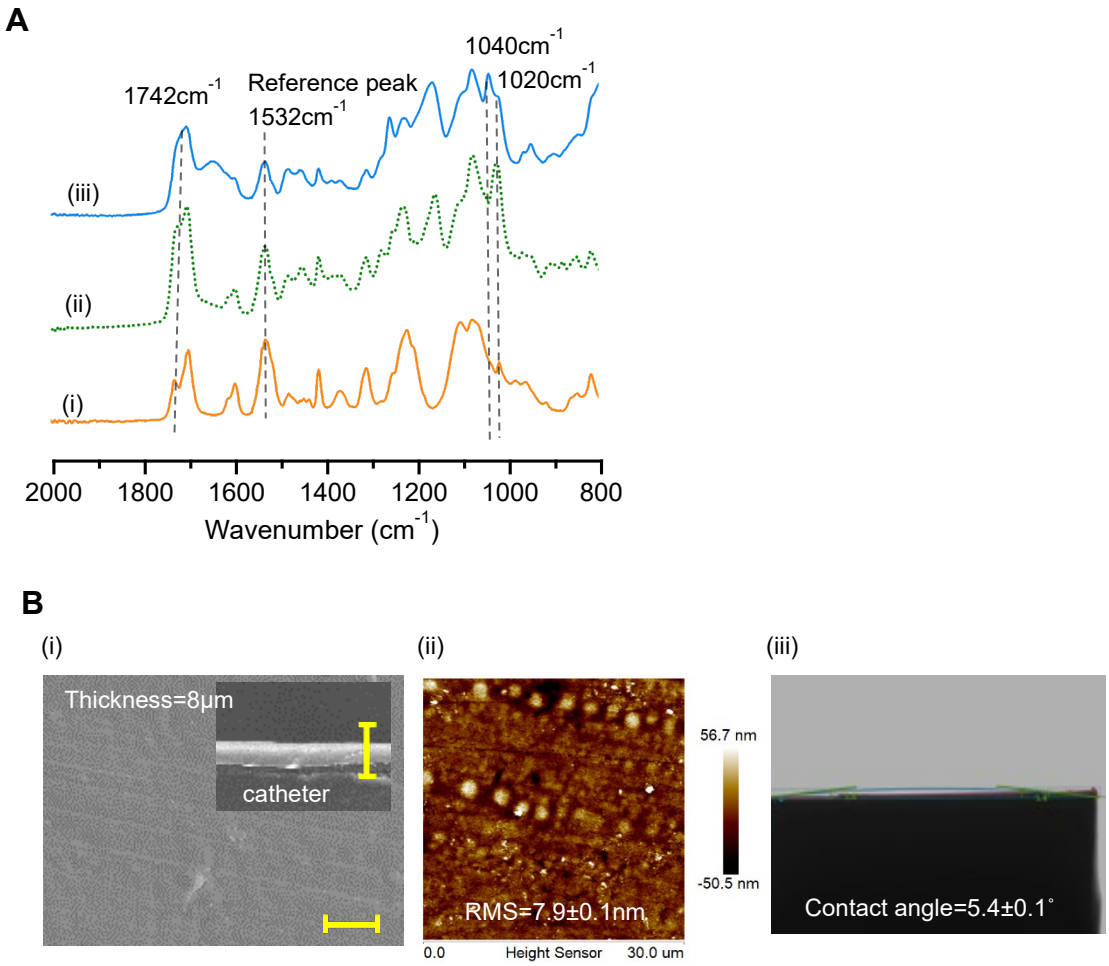
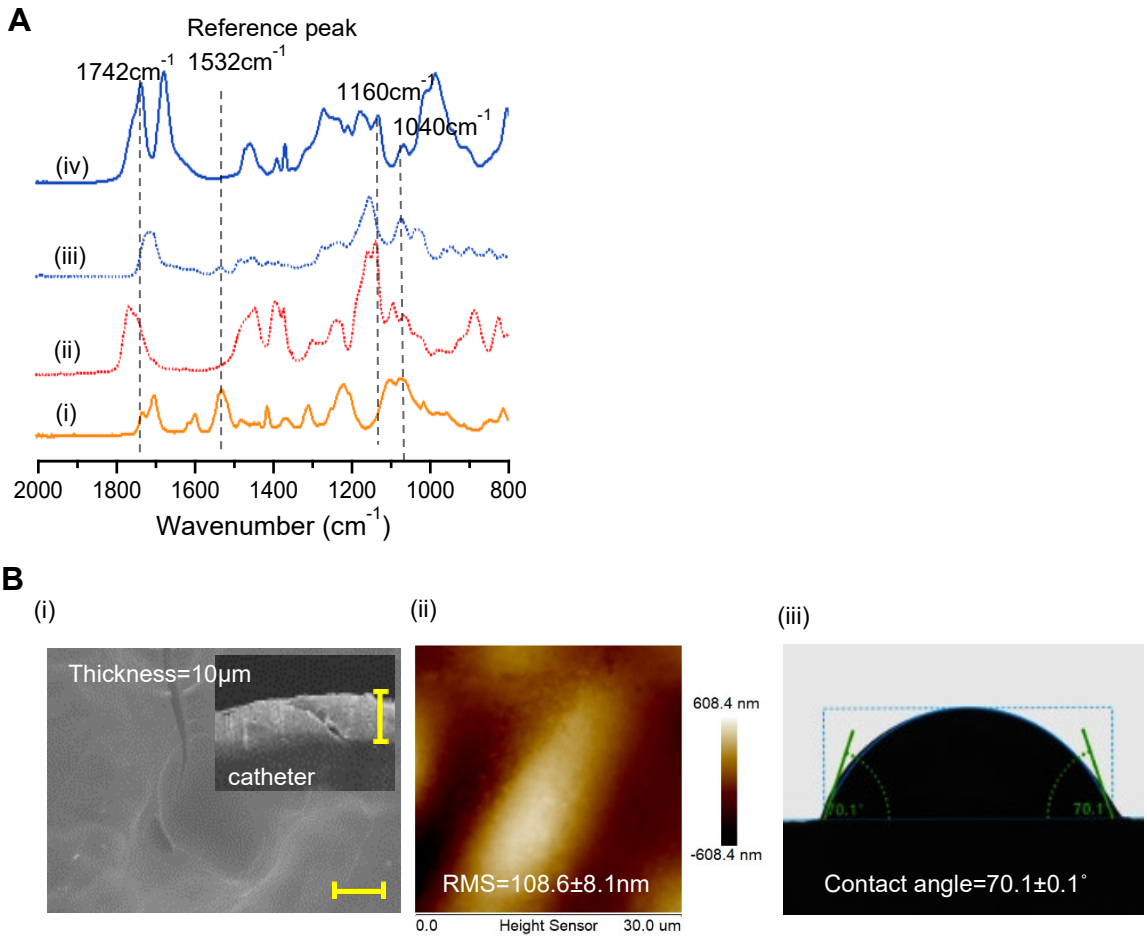


Figure S10. Characterization of coating (#7) H-*b*-S. **(A)** FTIR spectra of catheter samples: (i) unmodified control, (ii) poly(HEMA), (iii) (#7) H-*b*-S, with characterization peaks: C=O ester at 1742 cm^{-1}, SO_3^- sulfonyl peak at 1040 cm^{-1} and C-O-H peak at 1020 cm^{-1}. **(B)** (i) SEM image of catheter surface and cross section (inset) (scale bar=10 µm), (ii) AFM image with measured root mean square height variation, (iii) contact angle.


A
Reference peak
1742cm-1
1532cm-1
1160cm-1
1040cm-1
(iv)
(iii)
(ii)
(i)
2000 1800 1600 1400 1200 1000 800
Wavenumber (cm-1)
B
(i)
(ii)
(iii)
Thickness=10μm
catheter
608.4 nm
-608.4 nm
RMS=108.6±8.1nm
0.0 Height Sensor 30.0 um
70.1°
70.1°
Contact angle=70.1±0.1°
C
log10 absorbance at 341nm
NTMB-Cl (0.1mg/mL)
(#8) H(N)-x-S (24 hrs)
(#8) H(N)-x-S (1 week)
10
8
6
4
2
0
N.D
N.D
N.D
PBS
Methanol
Hexane

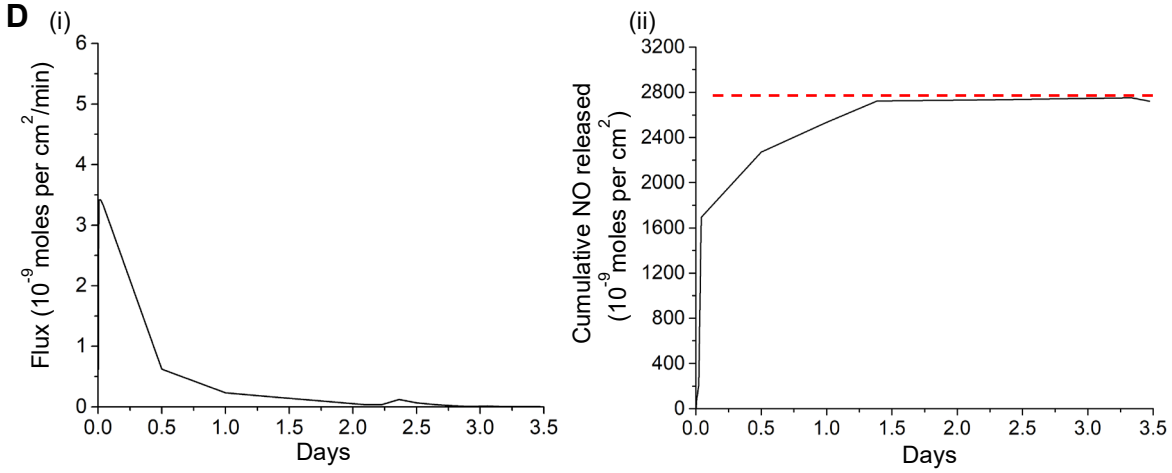
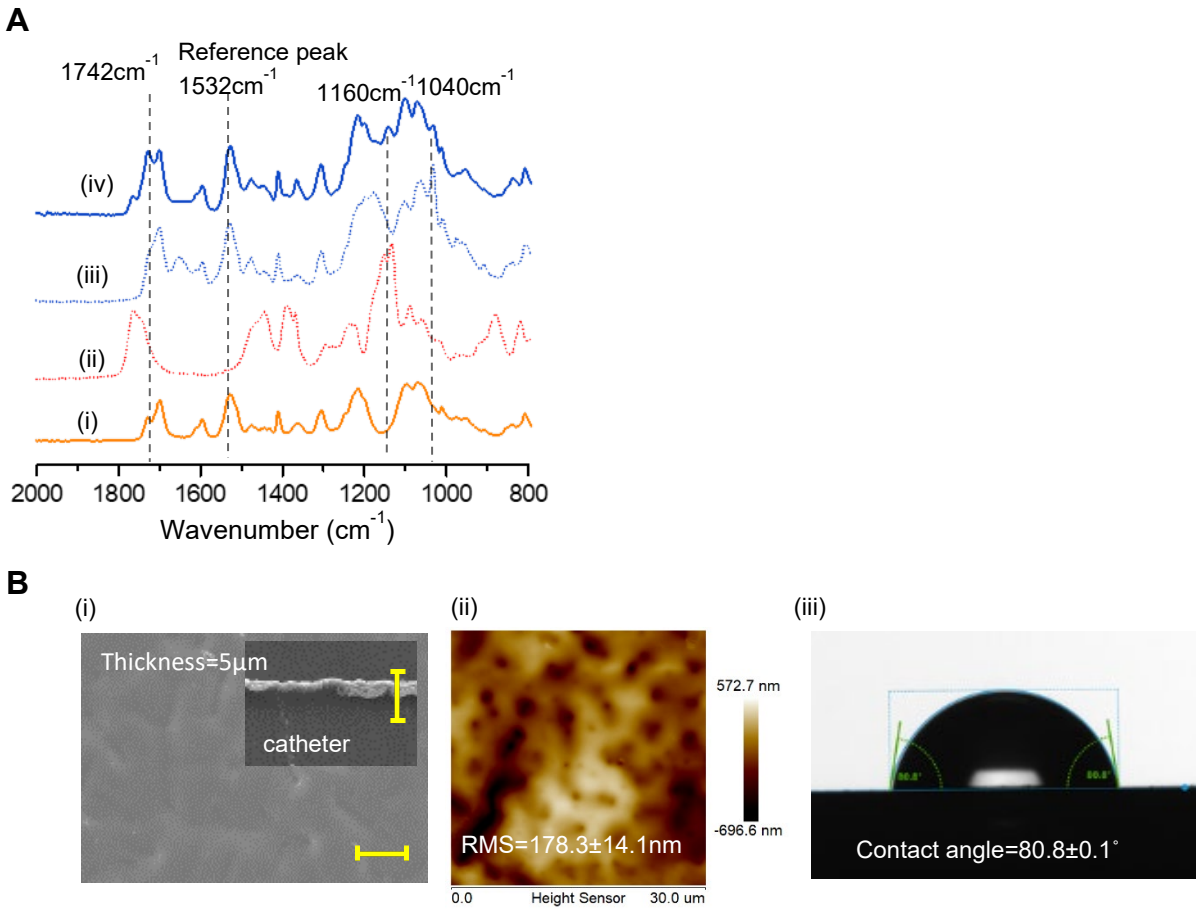


Figure S11. Characterization of coating (#8) H(N)-*x*-S. **(A)** FTIR spectra of catheter samples: (i) unmodified control, (ii) NO-donor NTMB-Cl, (iii) (#5) H-*x*-S, (iv) (#8) H(N)-*x*-S, characterization peaks: C=O ester at 1742 cm^{-1} and RSNO peak at 1160 cm^{-1} and SO_3^- sulfonyl peak at 1040 cm^{-1}. **(B)** (i) SEM image of catheter surface and cross section (inset) (scale bar=10 µm), (ii) AFM image with measured root mean square height variation, (iii) contact angle. **(C)** HPLC detection of NO release precursor (NTMB-Cl) leached to different solvents (N.D refers to no detection of leaching) in 24 h and 1 week extractions using PBS, methanol (polar solvent) and hexane (non-polar solvent). **(D)** NO flux measured at 55 ˚C, (ii) Cumulative NO released.


A
1742cm-1
Reference peak
1532cm-1
1160cm-1
1040cm-1
(iv)
(iii)
(ii)
(i)
2000
1800
1600
1400
1200
1000
800
Wavenumber (cm-1)
B
(i)
(ii)
(iii)
Thickness=5μm
catheter
RMS=178.3±14.1nm
572.7 nm
-696.6 nm
0.0
Height Sensor
30.0 um
Contact angle=80.8±0.1˚
C
log10 absorbance at 341nm
NTMB-Cl (0.1mg/mL)
(#8) H(N)-x-S (24 hrs)
(#8) H(N)-x-S (1 week)
N.D
N.D
N.D
PBS
Methanol
Hexane

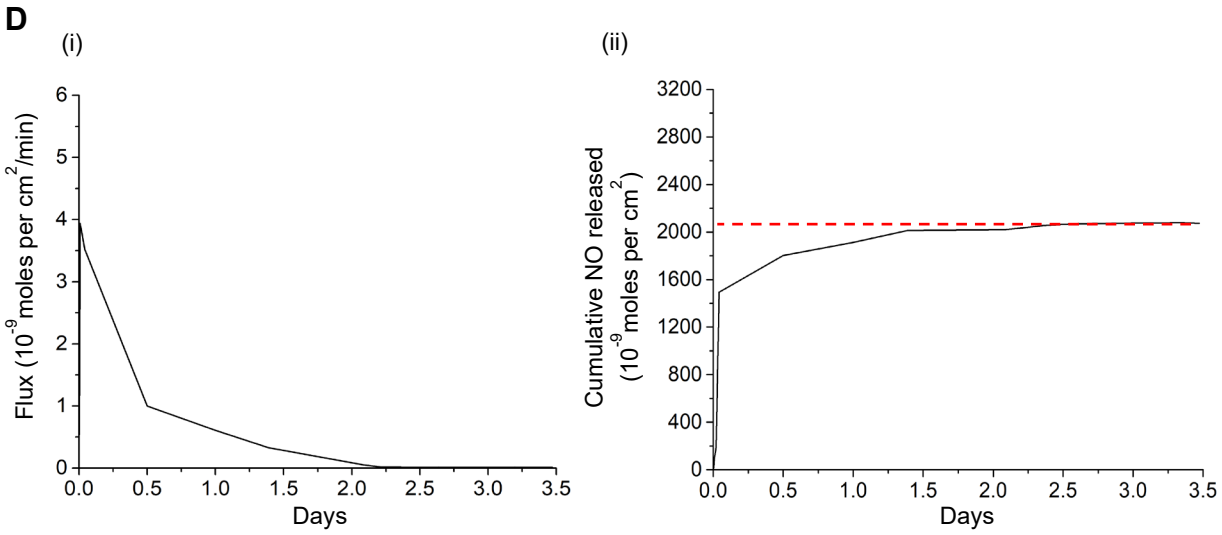


Figure S12. Characterization of coating (#9) H(N)-*r*-S. **(A)** FTIR spectra of catheter samples: (i) unmodified control, (ii) NO-donor NTMB-Cl, (iii) (#6) H-*r*-S, (iv) (#9) H(N)-*r*-S, characterization peaks: C=O ester at 1742 cm^{-1} and RSNO peak at 1160 cm^{-1} and SO_3^- sulfonyl peak at 1040 cm^{-1}. **(B)** (i) SEM image of catheter surface and cross section (inset) (scale bar=10 µm), (ii) AFM image with measured root mean square height variation, (iii) contact angle. **(C)** HPLC detection of NO release precursor (NTMB-Cl) leached to different solvents (N.D. refers to no detection of leaching) in 24 h and 1 week extractions using PBS, methanol (polar solvent) and hexane (non-polar solvent). **(D)** NO flux measured at 55 ˚C, (ii) Cumulative NO released.

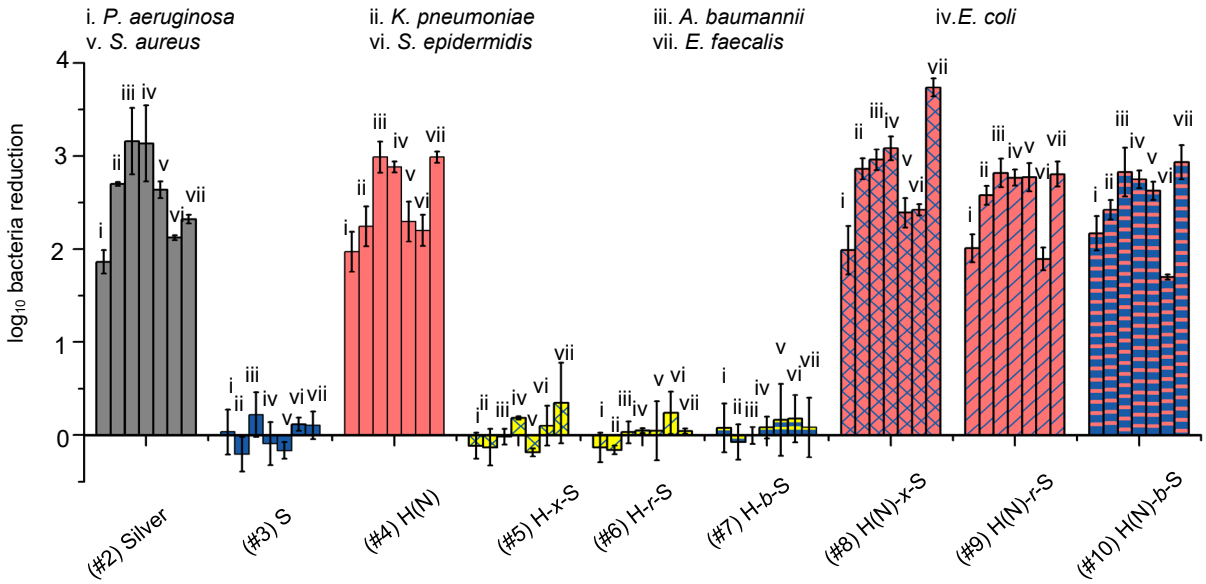
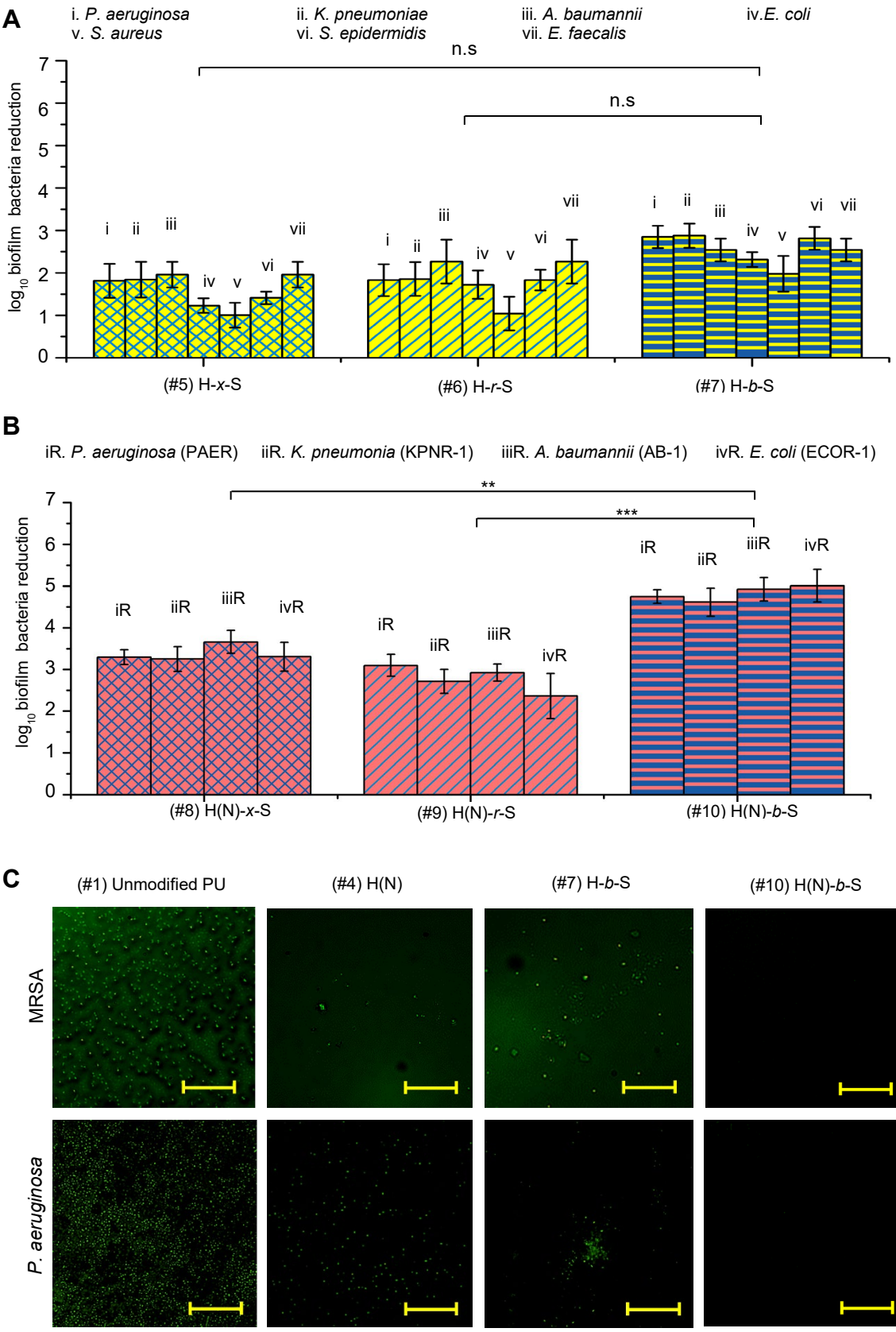


Figure S13. Acute (2 h) antimicrobial efficacy measured by contact killing with bacteria loaded on surface.


A
i. P. aeruginosa
ii. K. pneumoniae
iii. A. baumannii
iv.E. coli
v. S. aureus
vi. S. epidermidis
vii. E. faecalis
n.s
n.s
log10 biofilm bacteria reduction
(#5) H-x-S
(#6) H-r-S
(#7) H-b-S
B
iR. P. aeruginosa (PAER)
iiR. K. pneumonia (KPNR-1)
iiiR. A. baumannii (AB-1)
ivR. E. coli (ECOR-1)
**
***
log10 biofilm bacteria reduction
(#8) H(N)-x-S
(#9) H(N)-r-S
(#10) H(N)-b-S
C
(#1) Unmodified PU
(#4) H(N)
(#7) H-b-S
(#10) H(N)-b-S
MRSA
P. aeruginosa

D

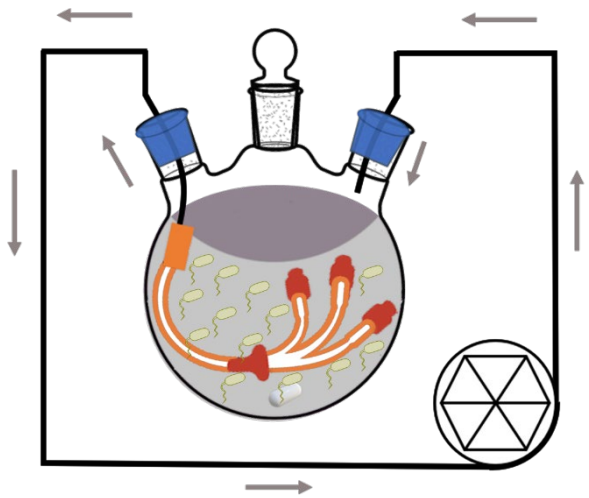


Figure S14. (A) 24 h *In vitro* antibiofilm efficacy of intermediate coatings (#5, #6 and #7) against some Gram-positive and Gram-negative bacteria. Student's *t*-test, n.s. $P>0.5$, **(B)** *In vitro* antibiofilm efficacy of NO-release coatings against multi-drug resistance (MDR) Gram-negative bacteria. Student's *t*-test, ***$P<0.001$, **$P<0.01$. **(C)** Fluorescence Microscopy of catheters incubated with MRSA and *P. aeruginosa* (scale bar=20 µm). **(D)** Illustration of intraluminal circulation setup for antibiofilm test.

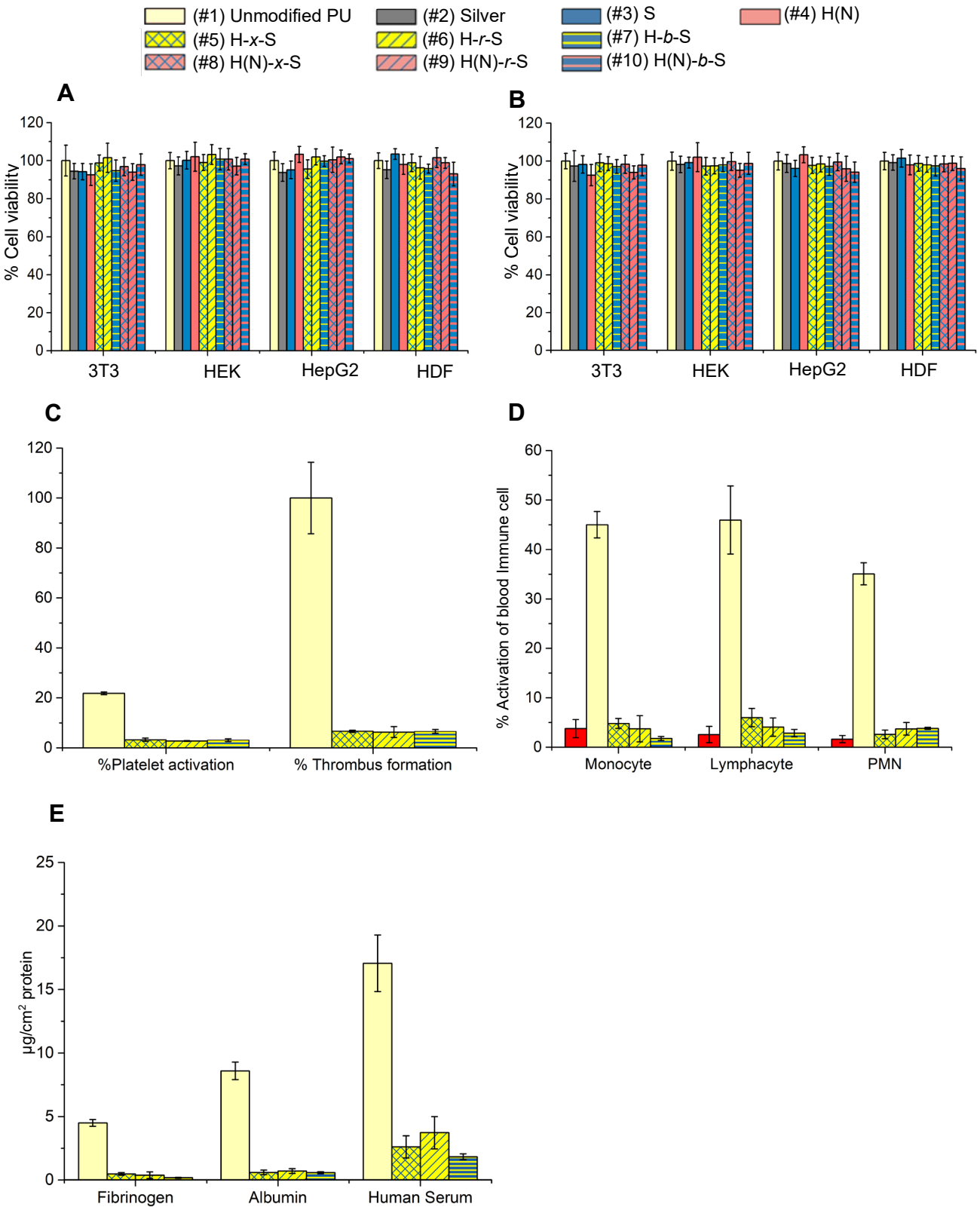


Figure S15. *In vitro* mammalian cell compatibility of extractants from modified catheters soaked in DMEM for **(A)** 24 h and **(B)** 72 h following ISO10993-5. **(C)** Hemocompatibility of intermediate catheters (#5, #6 and #7) measured by platelet activation and amount of thrombus formation. **(D)** Activation of blood immune cells. **(E)** Blood protein fouling on catheters after 24 h incubation with protein or serum.

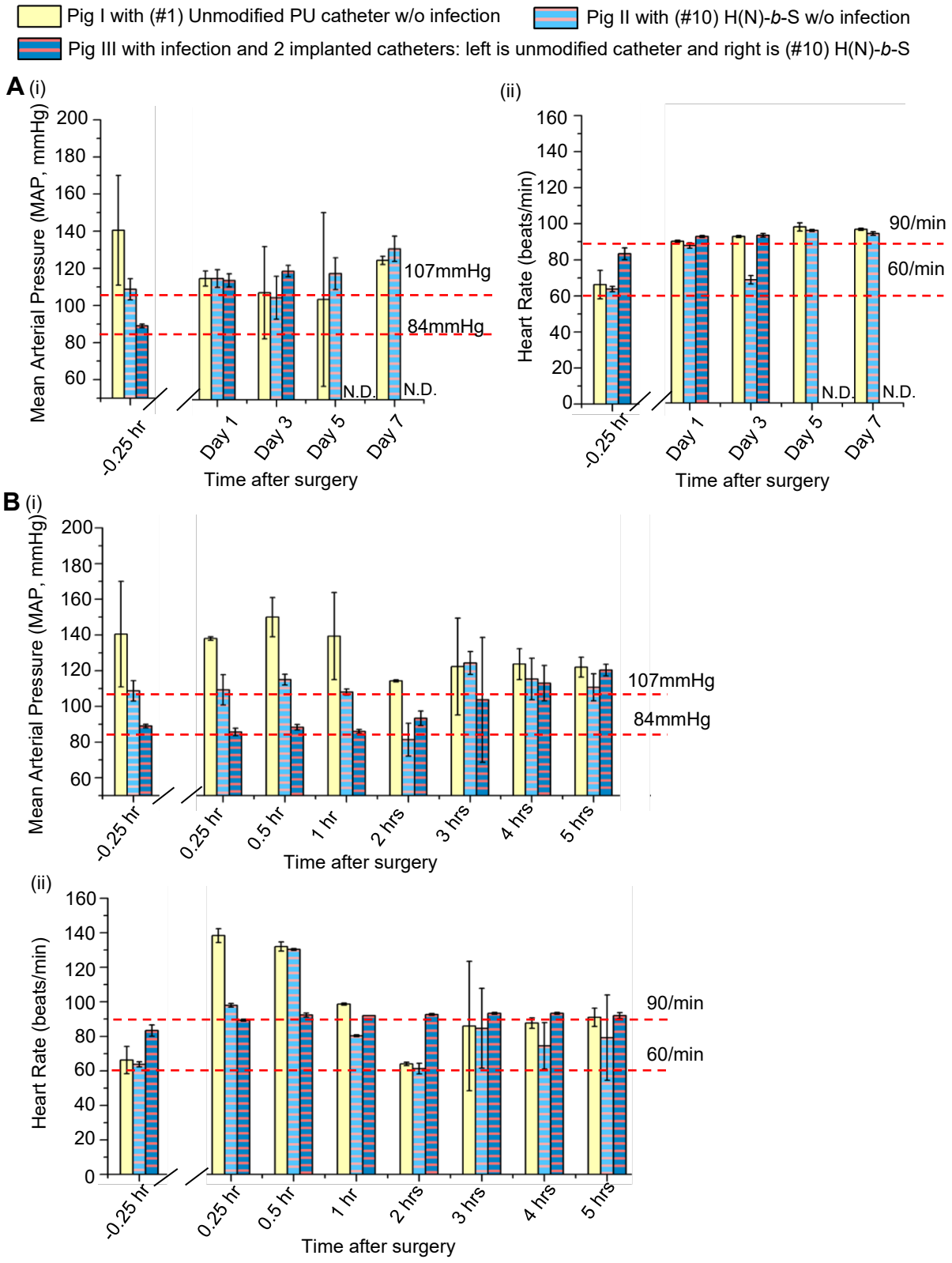


Figure S16. Monitoring of pigs' parameters (Time point of 0 is the point of completion of surgery to implant the catheter(s).). **(A)** Long-term observation (*i.e.* when the effects of anaesthesia are gone), (i) mean arterial pressure (MAP), (ii) heart rate (HR) (N.D. not done because infected pig was sacrificed on Day 5). **(B)** Transient observation after surgery and until full wake-up from anaesthesia, (i) mean arterial pressure (MAP), (ii) heart rate (HR).

Equation S1. Surface peroxide group density

Volume of Sodium thiosulfate solution (0.01 mM) used: 3.30 mL

Moles of peroxide equals moles of thiosulfate titrated, calculated as below:

$$0.01 \times 10^{-3} \times 3.30 \times 10^{-3} = 3.3 \times 10^{-8}\ mole\ of\ peroxide\ per\ 5\ mm\ of\ catheter$$

Calculation of peroxide group density (σ):

$$\sigma = \frac{No.\ of\ peroxide\ group\ on\ 5\ mm\ of\ catheter}{Surface\ area\ of\ 5\ mm\ catheter}$$

$$\sigma = \frac{3.3 \times 10^{-8} \times 6.023 \times 10^{23}}{0.25 \times 0.5 \times \pi + 0.4 \times 0.5 \times \pi + 2 \times ((0.5 \times 0.4)^2 - (0.5 \times 0.25)^2) \times \pi}$$

$$\sigma = \frac{1.98 \times 10^{16}}{1.17\ cm^2}$$

$$\sigma = 1.69 \times 10^{16}\ /cm^2$$

$$\sigma = 169\ /nm^2$$