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Supporting Information

Precisely Structured NO-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)

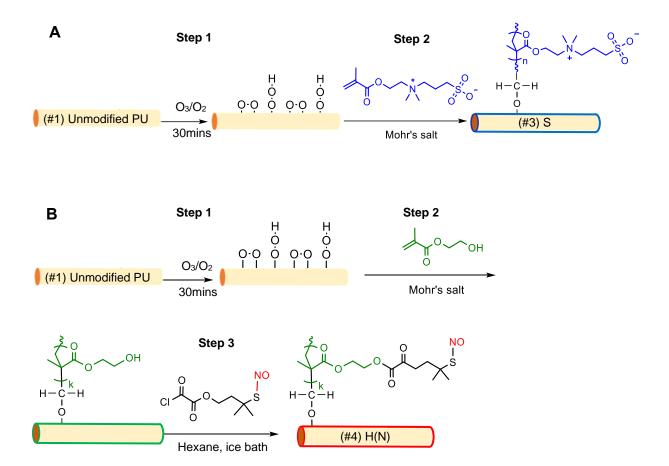


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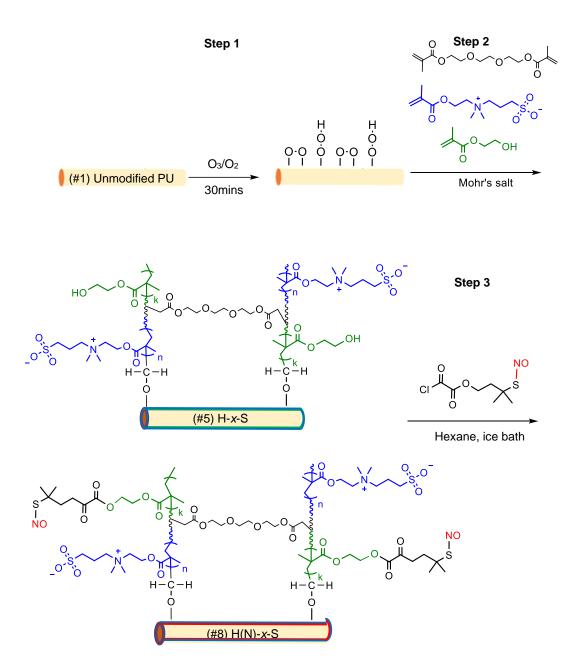


Figure S2. Synthesis of crosslinked coating ((#5) H-x-S and (#8) H(N)-x-S).

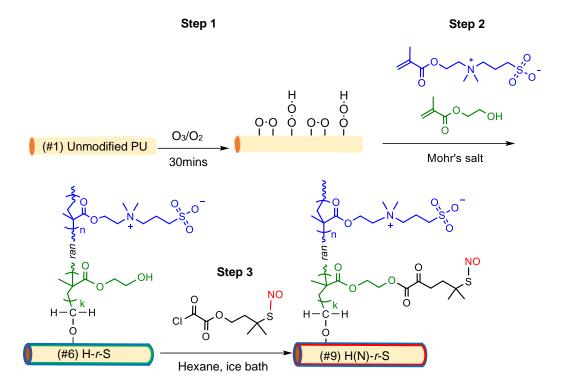


Figure S3. Synthesis of random copolymer coating ((#6) H-*r*-S and (#9) H(N)-*r*-S).

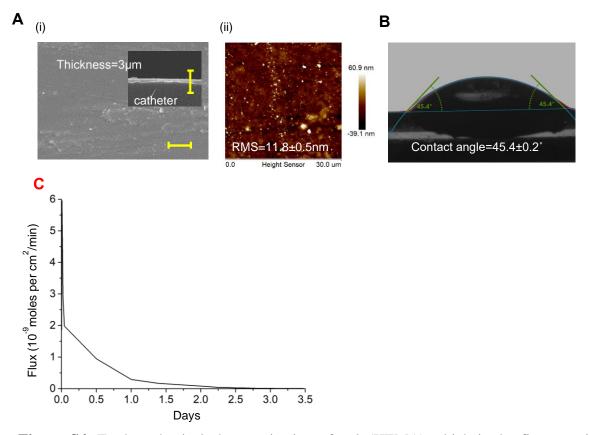


Figure S4. Further physical characterization of poly(HEMA) which is the first step in the synthesis of (#10) H(N)-*b*-S. (**A**) Surface morphology (i) SEM image of catheter surface and cross section (inset) (scale bar=10μm), (ii) AFM image of surface morphology with measured root mean square height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

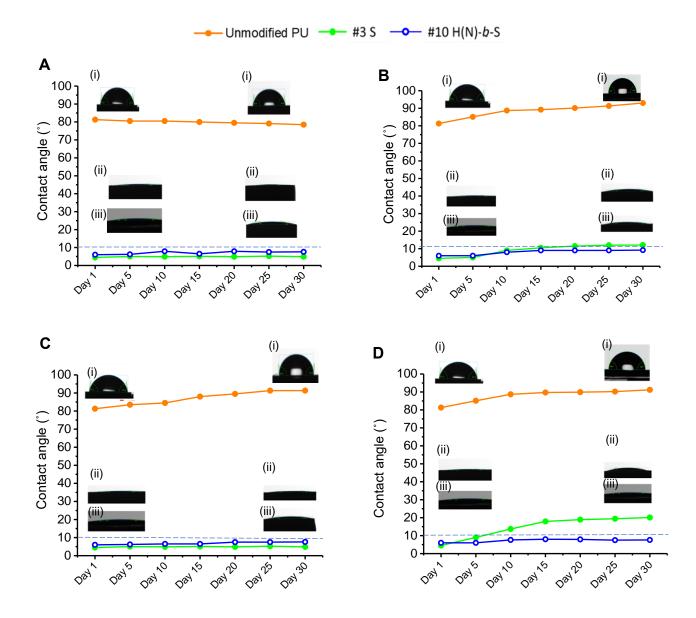
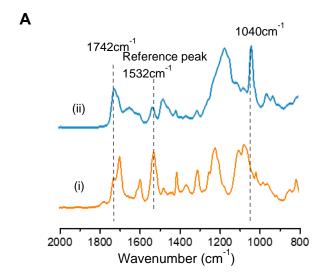


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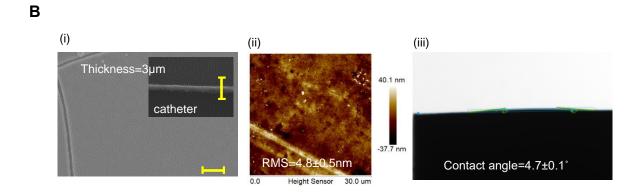


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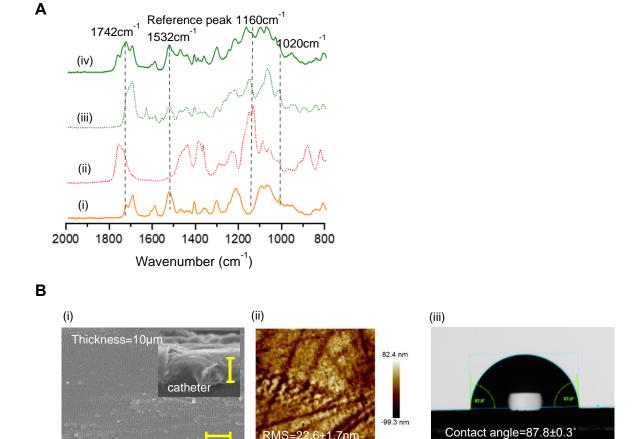


Figure S7. Characterization of coating (#4) H(N). (**A**) FTIR spectra of catheter samples: (i) unmodified control, (ii) NO-donor NTMB-Cl, (iii) homo poly(HEMA) coating, (iv) (#3) H(N) coating with characterization peaks: C=O ester at 1742cm⁻¹ and RSNO peak at 1160cm⁻¹. (**B**) (i) SEM image of catheter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

Height Sensor

30.0 um

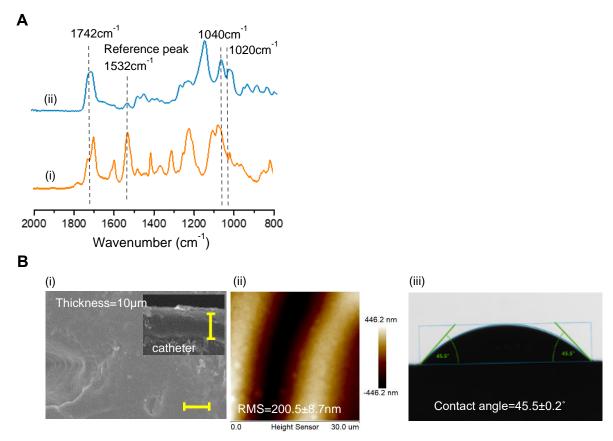
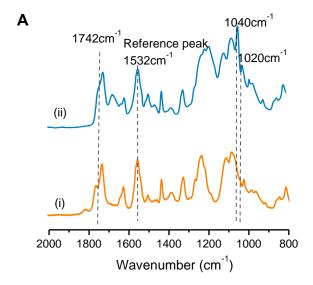


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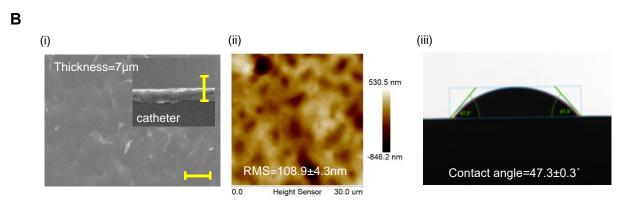


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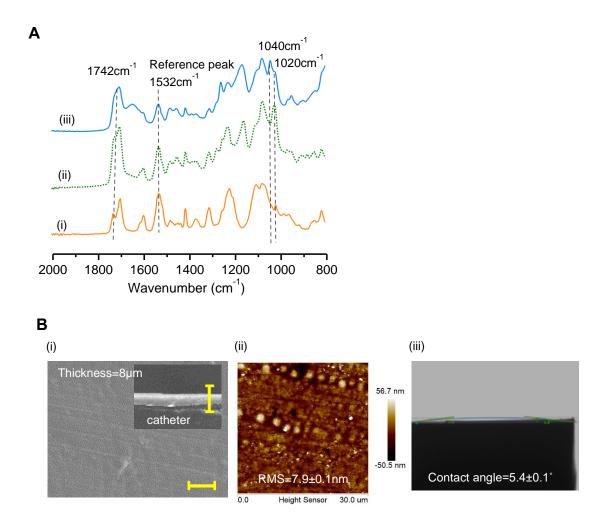


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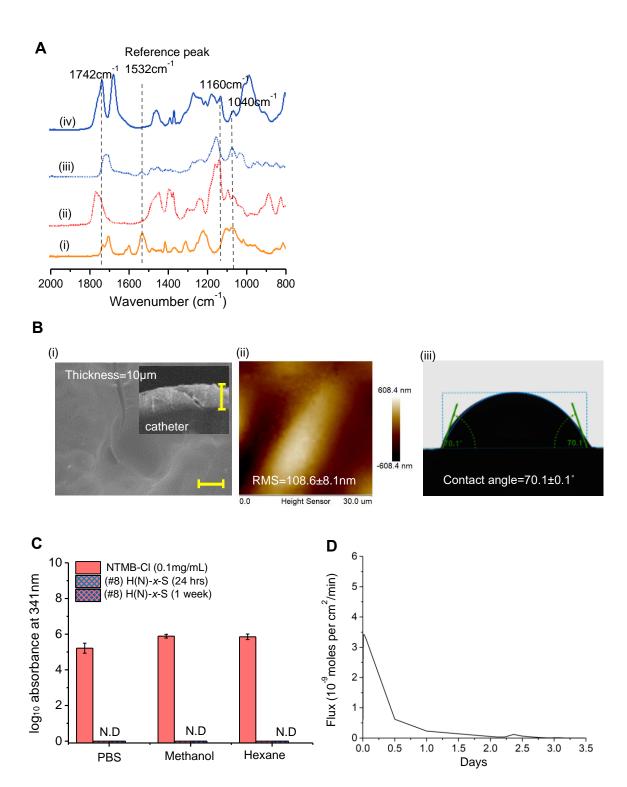


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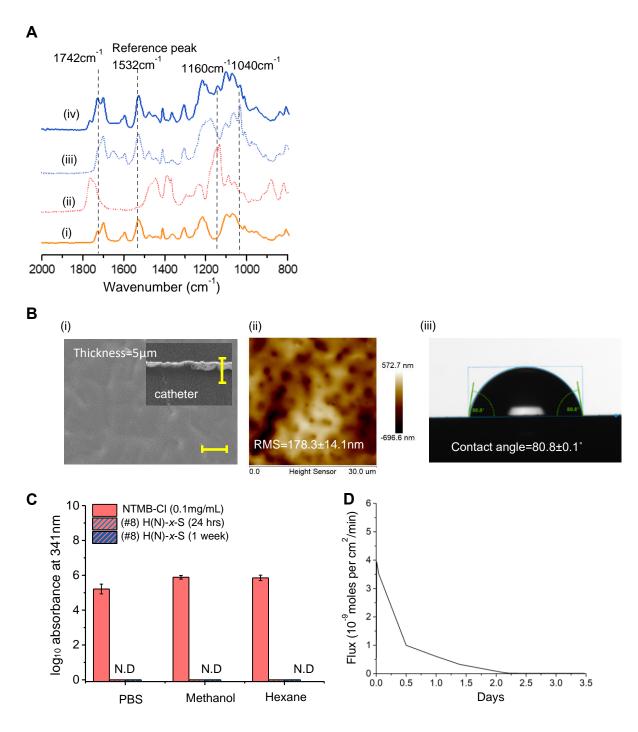


Figure S12. Characterization of coating (#9) H(N)-*r*-S. (**A**) FTIR spectra of catheter samples: (i) unmodified control, (ii) NO-donor NTMB-Cl, (iii) (#6) H-*r*-S, (iv) (#9) H(N)-*r*-S, characterization peaks: C=O ester at 1742cm⁻¹ and RSNO peak at 1160cm⁻¹ and SO₃⁻ sulfonyl peak at 1040cm⁻¹. (**B**) (i) SEM image of catheter surface and cross section (inset) (scale bar=10μm), (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 24hr and 1week extractions using PBS, methanol (polar solvent) and hexane (non-polar solvent). (**D**) NO flux measured at 55°C.

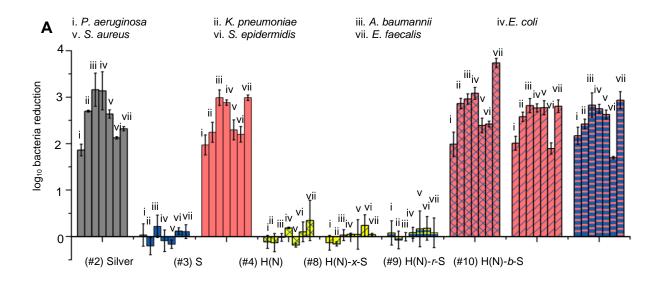
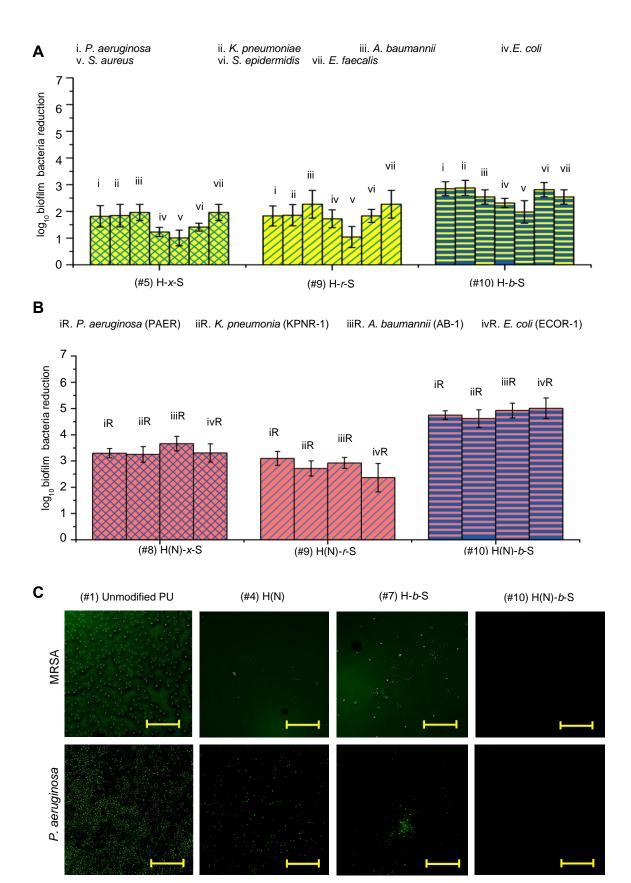


Figure S13 Acute (2-hr) antimicrobial efficacy measured by contact killing with bacteria loaded on surface.



D

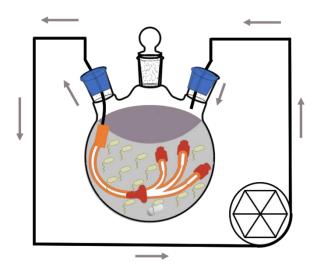


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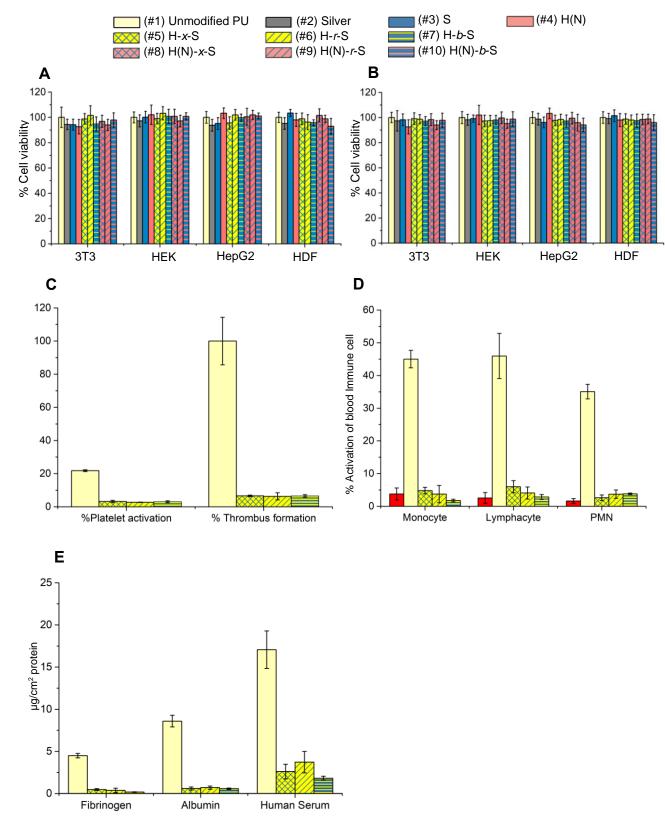


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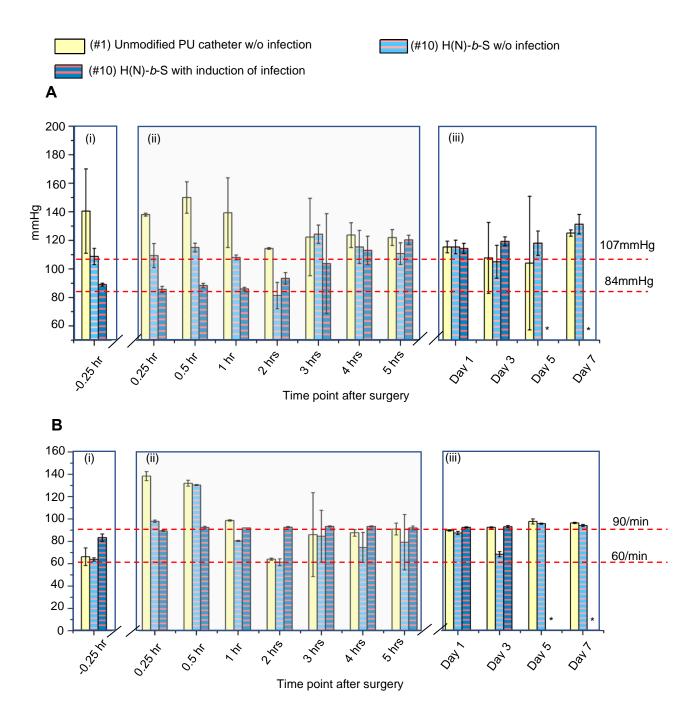


Figure S16. Monitoring of pig test subjects: (A) mean arterial pressure and ((i) before implantation and under anaesthesia, (ii) After implantation and until full wake-up from anaesthesia, (iii) Without anaesthesia) (B) heart rate in porcine central venous implantation of uninfected pigs. ((i) before implantation and under anaesthesia, (ii) After implantation and until full wake-up from anaesthesia, (iii) Without anaesthesia) (*infected pig was sacrificed on Day 5).

Equation S1 Surface peroxide group density

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

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 5mm of catheter Calculation of peroxide group density (σ):

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

$$\sigma = \frac{3.3\times10^{-8}\times6.023\times10^{23}}{0.25\times0.5\times\pi+0.4\times0.5\times\pi+2\times((0.5\times0.4)^2-(0.5\times0.25)^2)\times\pi}$$

$$\sigma = \frac{1.98\times10^{16}}{1.17cm^2}$$

$$\sigma = 1.69\times10^{16}/cm^2$$

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