Supplementary Information for

Antithrombotic and antimicrobial potential of *S*-nitroso-1-adamantanethiol-impregnated extracorporeal circuit

Authors

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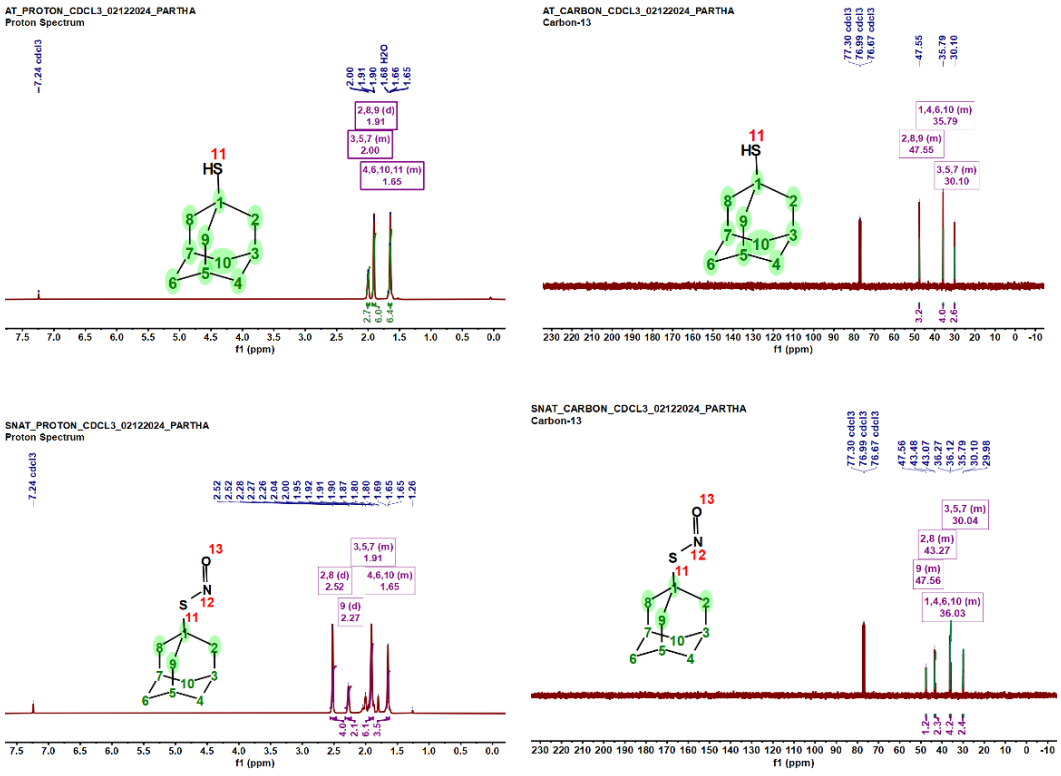

Figure S1. ^{1}H (left) and ^{13}C (right) spectra of 1-adamantanethiol (AT, top) and S-nitroso-1-adamantanethiol (SNAT, bottom)

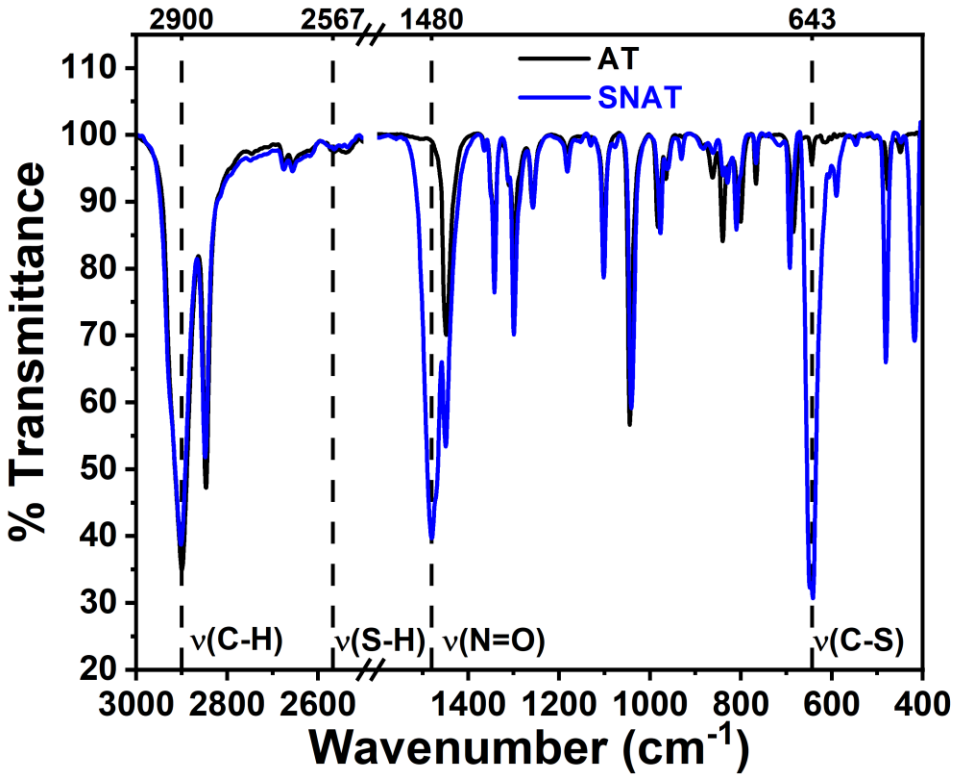

Figure S2. ATR-FTIR spectrum of S-nitroso-1-adamantanethiol (SNAT, blue) and 1-adamantanethiol (AT, black) molecules

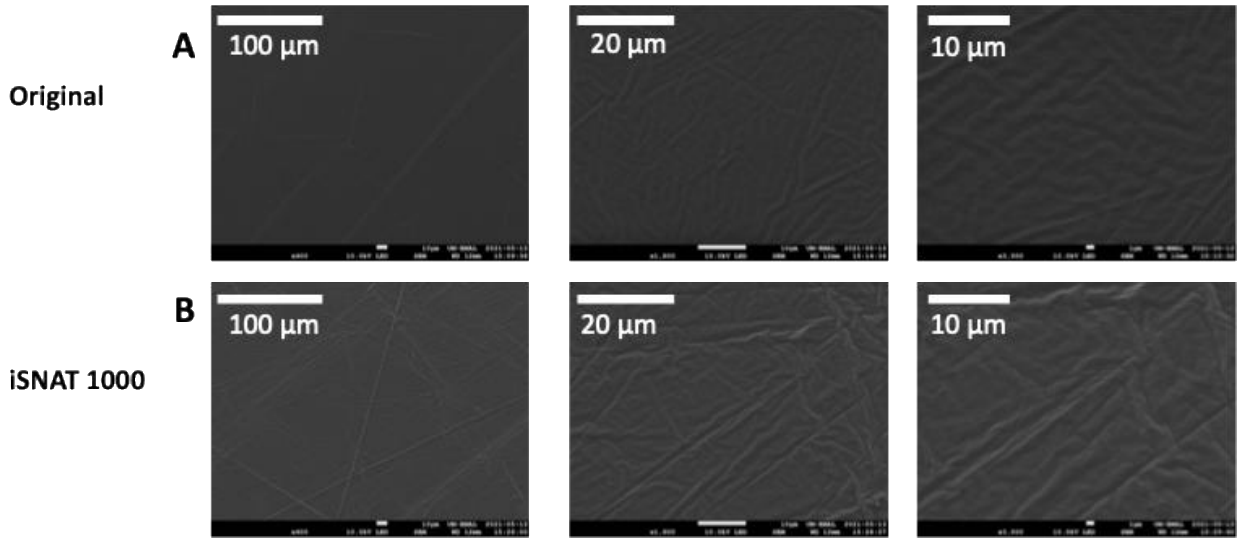

Figure S3. Scanning Electron Micrographs of the untreated, naïve (**A**), and the iSNAT 1000 (**B**) impregnated PVC surface with different magnifications. Scale bars are added to the top left corners of the micrographs.

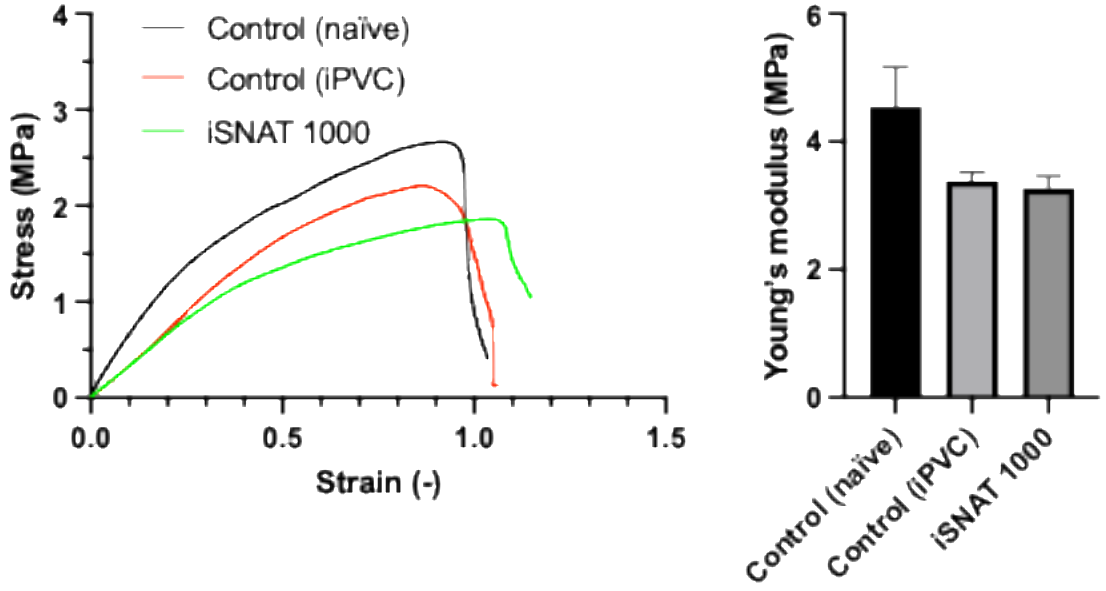

Figure S4. Representative stress-strain curve (left), and the comparison of Young's modulus for the naïve (non-impregnated), iPVC (solvent impregnated without SNAT) and iSNAT 1000 impregnated PVC (right, n=4, Data±SEM, $p<0.05$) samples.

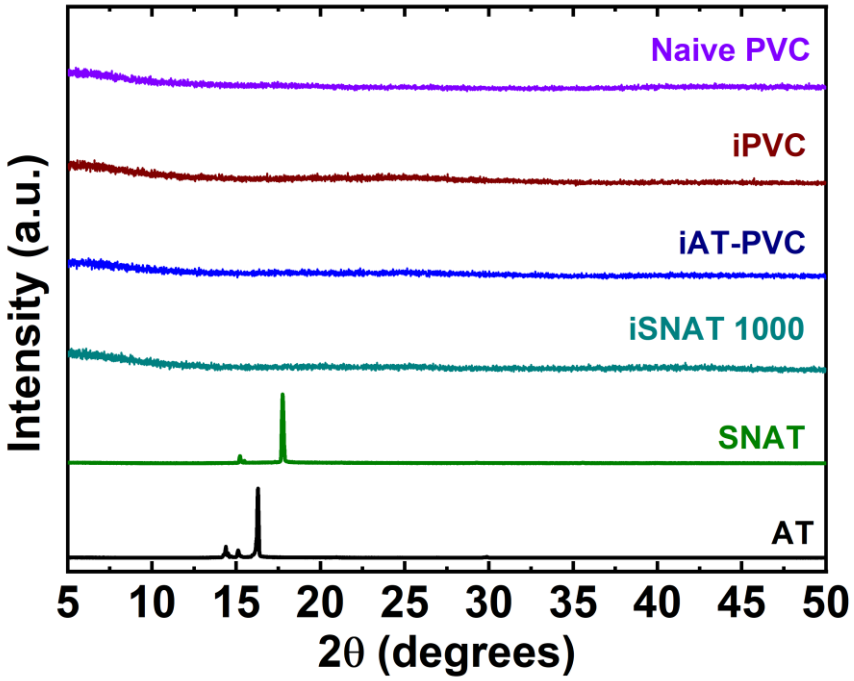

Figure S5. Comparison of the PXRD patterns of naïve (non-impregnated), iPVC (solvent impregnated without SNAT), iAT-PVC (1-adamantanethiol impregnated), iSNAT 1000 (SNAT impregnated) PVC samples, and the powdered SNAT (S-nitroso-1-adamantanethiol) and AT (1-adamantanethiol).

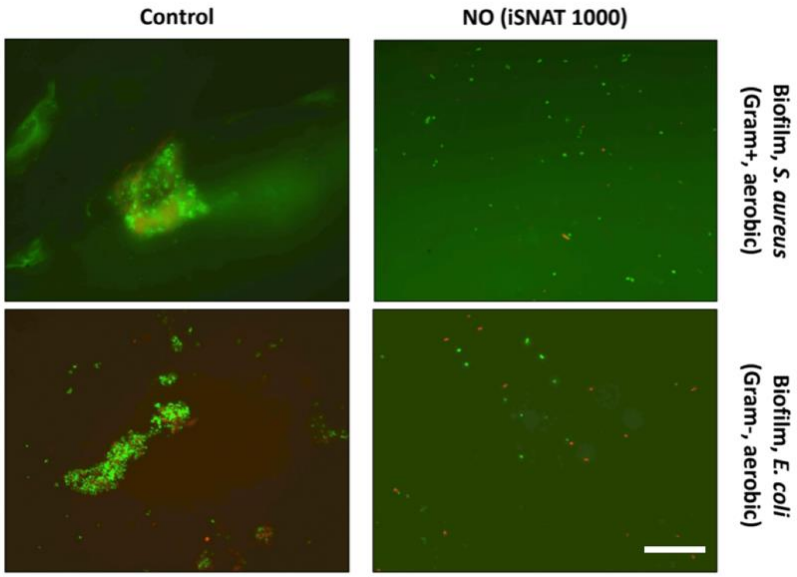

Figure S6. Representative microscopy images of the iSNAT 1000 antimicrobial effect on biofilm formation using *S. aureus* and *E. coli* strains compared to the naïve control, live cells-green, dead cells-red, scale bar represents length of 20 μm.

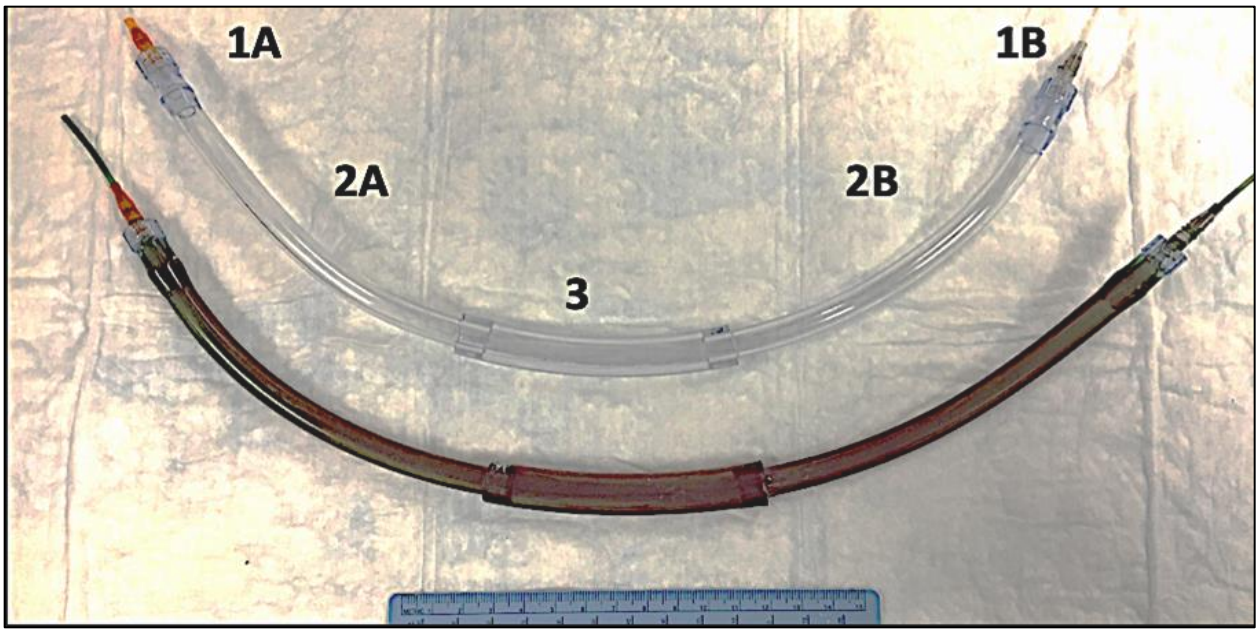

Figure S7. The untreated control (clear, translucent; top) and the 1000 mg/mL SNAT (green, translucent; bottom) impregnated PVC circuit (pre-surgery) with angiocatheters (1A, 1B) at the end, the ECC loop (2A, 2B, ¼" ID ND 100 65 Tygon, PVC) and the thrombogenicity chamber (3, 3/8" ND-100-65 Tygon, PVC).

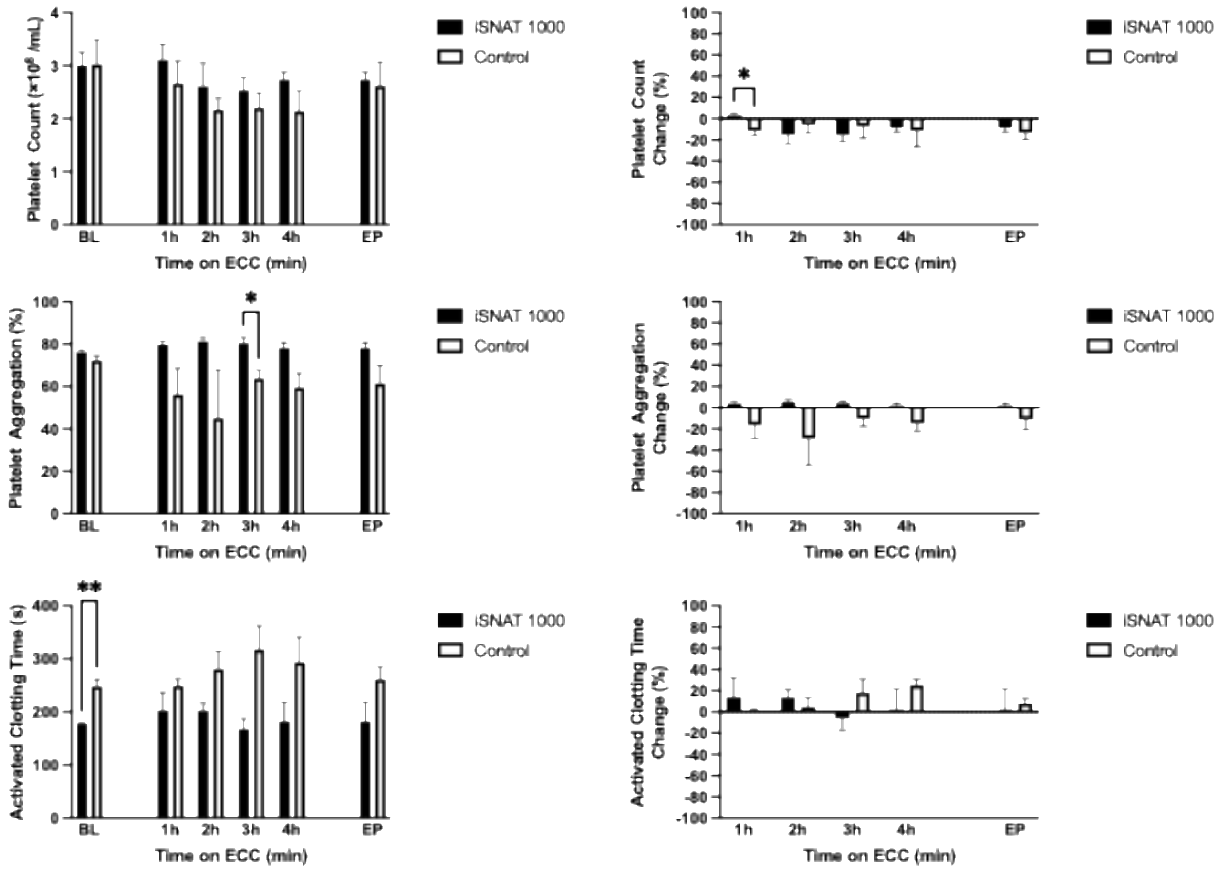

Figure S8. Platelet count (A), platelet aggregation (B) and activated clotting time values (C) (left) and the corresponding changes relative to the baseline (right) of the iSNAT 1000 impregnated (black bar) vs. untreated (naïve) control (white bar) ECC groups over 4 hours. BL: baseline; EP: end point, which was 4.0±0.0 hour in the iSNAT 1000 and 2.3±0.6 hour in the Control groups; Data are mean ± SEM; significance (P≤0.05) is labeled with asterisk and bracket.

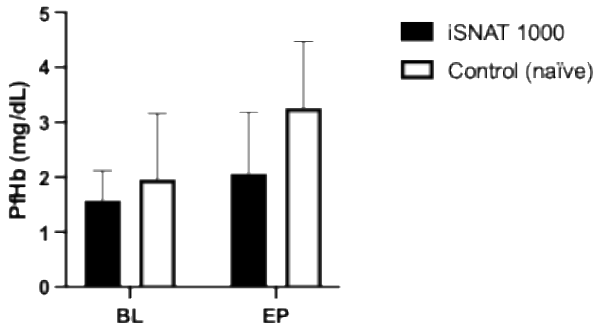

Figure S9. Plasma free hemoglobin (PfHb) levels. BL: baseline; EP: end point, which was 4.0±0.0 hour in the iSNAT 1000 and 2.3±0.6 hour in the Control groups; error bars represent SEM; significance (P≤0.05) is labeled with asterisk and bracket.

Methodology for NMR measurement

NMR spectra for ^{1}H and ^{13}C nuclei of AT and SNAT were obtained at 21°C by dissolving the solid compounds in deuterated chloroform ($CDCl_3$). Chemical shifts for ^{1}H and ^{13}C NMR spectra were calibrated in parts per million (ppm) relative to tetramethylsilane (TMS) as an external standard, with internal referencing to the solvent's residual proton signals. These spectra were acquired using a Varian 400 MHz NMR spectrometer and analyzed with MestReNova software.

Methodology for ATR-FTIR measurement

ATR-FTIR spectroscopy analysis for solid AT and SNAT was conducted using an FT/IR-4X Spectrometer equipped with an ATR Pro 4X (Jasco, Inc., Japan). Prior to sample placement, a background spectrum was recorded in the air. The spectra of the solid powder samples were then acquired within a wavenumber range of 400 cm^{-1} to 4000 cm^{-1} at 21°C. The same experimental parameters were applied for both background and

sample spectra recordings, with the background spectrum subtracted from the sample spectra to ensure measurement accuracy.

Methodology for the Powder X-ray Diffraction (PXRD) measurements

PXRD measurements of naïve PVC, solvent impregnated PVC without NO donor (iPVC), AT impregnated PVC (iAT-PVC) and iSNAT 1000 impregnated PVC, powdered SNAT and AT were performed according to the methodology described by Wo *et al*.:[1] Patterns were collected at room temperature using a Rigaku R-Axis Spider diffractometer with an image plate detector and graphite monochromated Cu-Kα radiation (λ = 1.54187 Å) at 40 kV and 44 mA. SNAT and AT samples were finely ground to eliminate preferred orientation, whereas PVC samples were cut into 250 µm cubes. All samples were mounted on a CryoLoop using heavy mineral oil, and images were collected for 15 min with a 0.3 mm collimator. The ω-axis was oscillated between 120° and 180° at 1°/s, the ϕ-axis was rotated at 10°/s, and the χ-axis was fixed at 45°.

Methodology for the *in vitro* antimicrobial assay

To test the antimicrobial efficacy, we followed the methodology from Colletta *et al*.[2] and Brisbois *et al*.[3] 3.06 cm^2 surface area tubing samples were employed. All experiments were conducted in at least triplicate, and the results were compared to the unmodified control group.

For the biofilm study the two tested bacterial strains (*Staphylococcus aureus* ATCC25923 and *Escherichia coli* K12) were maintained on a Luria Bertani (LB) agar plate and grown in LB broth. Biofilms were developed in a CDC biofilm reactor (BioSurface Technologies,

Bozeman, MT, USA) supplemented with 10% strength of LB broth. The total volume of media was 400 mL and the surface area to total volume ratio (SA:V) was 0.00765 cm^2/mL. Four mL of overnight-grown bacterial culture were inoculated into the CDC biofilm reactor at a final concentration of about 10^6 colony-forming units (CFU)/mL, and the CDC biofilm reactor was left static for 1 h before introducing fresh 10% LB media at 100 mL/h via a peristaltic pump and starting the magnetic stirrer to generate shear force (300 rpm, ~0.08 N m^{-2}).[4] The biofilms were allowed to develop on the surface of the iSNAT (n=4) and control (n=4) polymer samples in separate bioreactor for 7 days at 37°C to avoid any cross contamination. The samples were then taken out of the reactor aseptically and gently rinsed in sterile PBS to remove any loosely attached bacterial cells. One of the samples from each group was stained with a BacLight Live(green)/Dead(red) staining kit (L7012, Thermo Fisher Scientific, Ann Arbor, MI) in the dark for 15 min to assess the degree of biofilm formation. Microscopic images were obtained by using a fluorescence microscope with appropriate filter sets (488/520 nm for SYTO-9 and 493/636 nm for propidium iodide) and x60 lens. The other pieces were transferred into sterile PBS buffer and were then homogenized to form a homogeneous cell suspension. Cell suspensions were serially diluted and plated onto LB agar plates to assess cell viability. All experiments were conducted in at least triplicate.

For testing the bacterial killing effect, the bacteria of three strains, *Pseudomonas aeruginosa* PAO1, *S. aureus* ATCC25923, and *E. coli* K12 were used separately. They were grown overnight in 10% LB broth at a final concentration of $1x10^6$ CFU/mL. The samples (n=3 iSNAT and n=3 unmodified control) were incubated at 37°C with gentle shaking (80 rpm) for 5 days in separate bioreactors without continuous media

supplementation. After incubation, the samples were carefully removed, and the remaining bacterial culture (planktonic cell) was diluted 10-fold in PBS buffer and was used for plate counting.

Methodology of the *in vivo* thrombogenicity model

The live rabbit arteriovenous (A/V) shunt model for thrombogenicity has been standard in our lab for many years.[5] Although there are many methods to assess thrombogenicity of materials exposed to blood (SEM, contact angle, electric charge, etc.) we believe the definitive screening test requires exposure to warm fresh blood from a live animal. All animal experiments were performed following the National Institutes of Health's Guide for the Care and Use of Laboratory Animals. This study was approved by the Institutional Animal Care & Use Committee (IACUC) at the University of Michigan (PRO00008843). Components of the custom-built ECC were glued together by using a 60 mg/mL CarboSil®/tetrahydrofuran (THF) (Sigma Aldrich) solution. The ECC circuit consisted of a 16G and a 14G polyurethane IV angiocatheter (Kendall Monoject Tyco Healthcare, Mansfield, MA), two 14.5 cm long ¼" ID PVC tubings (Tygon ND-100-65, Fisher Healthcare, Houston, TX), and one 9 cm long ⅜" ID PVC thrombogenicity chamber (Tygon ND-100-65, Fisher Healthcare, Houston, TX) in the middle. The angiocatheters were connected with tubing using PVC connectors (Qosina Corp., Ronkonkoma, NY) on both ends of the circuit. The impregnated circuit components were primed with physiological saline solution on the day of surgery, before implantation in the animal. Ten male SPF New Zealand white rabbits weighing 2.0-3.0 kg (Robinson Services Incorporated, Illinois) were tested (n=7 control, n=3 NO). No systemic anticoagulation

was used during the experiments. Rabbit whole blood was collected in an anticoagulated (3.2% sodium citrate, Vacutainer®, Becton Dickinson, Franklin Lakes, NJ) tube at baseline (prior to placement of the A/V extracorporeal circuit), and at the 1 h, 2 h, 3 h and 4 h time points after connection to the iSNAT or naïve control circuits. The main hemodynamic parameters (pH, PCO_2, PO_2, total hemoglobin, methemoglobin) were measured with an ABL 800 FLEX blood-gas analyzer (Radiometer, Copenhagen, DK). The platelet and total white blood cell (WBC) counts were also monitored and measured by IDEXX ProCyte Dx (IDEXX Laboratories, Westbrook, ME), the activated clotting time (ACT) by the Hemochron Blood Coagulation System (Model 801, International Technidyne Corp. Edison, NJ) using non-anticoagulated whole blood. The platelet function was assessed via a Chrono-Log optical (turbidimetric) aggregometer (model 490, Havertown, PA) using platelet rich plasma (PRP) according to a published method, where the percentage of aggregation was determined 3 min after the addition of collagen.[5] The degree of hemolysis (level of plasma-free hemoglobin) was also evaluated from platelet poor plasma (PPP) via a UV spectrophotometer (Ultrospec 500 pro Spectrophotometer, Amersham Biosciences, United Kingdom) using the previously described automated Harboe method.[6] The concentration of plasma free hemoglobin was calculated using the following equation: fHb (mg/dL) = 83.6 (2x A415nm – A380nm – A450nm).[6]

Data collected after baseline (BL) were corrected for hemodilution due to IV fluid administration. The endpoints (EP) of the studies were at 4 h on ECC or when the flow rate in the ECC dropped to zero mL/min due to occlusive clot formation, whichever was earlier. At EP, all animals received a dose of 400 U/kg sodium heparin to prevent necrotic thrombosis, then the animals were euthanized. After that the thrombogenicity chamber of

the ECC was analyzed for any sign of thrombus formation. A photograph of the chamber was taken, and the thrombus area was further quantified using ImageJ software (National Institutes of Health, Bethesda, MD).

References cited in the Supplementary information

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