

Supporting Information

Electrochemically Modulated Nitric Oxide (NO) Releasing Biomedical Devices via Cu(II)-Tri(2-pyridylmethyl)amine Mediated Reduction of Nitrite

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EXPERIMENTAL DETAILS

Materials:

Sodium nitrite, sodium chloride, sodium phosphate dibasic (99.95%), sodium phosphate monohydrate (99.5%), 3-(N-morpholino)propanesulfonic acid (99.5%), 3-(N-morpholino)propanesulfonic acid sodium salt (99.5%), copper(II) acetate monohydrate (99.99%), and tri(2-pyridylmethyl)amine (98%) were purchased from Sigma-Aldrich (St. Louis, MO) and used as received.

PTFE coated platinum (0.125 mm) and silver (0.127 mm) wires were purchase from Sigmund Cohn Corp. (Mount Vernon, NY). Single lumen standard silicone tubing (O.D. 1.47 mm, i.d. 1.96 mm) was purchased from Helix Medical (Carpinteria, CA) while dual lumen silicone catheters (o.d. 2.34 mm) were provided by Cook Medical (Bloomington, IN).

Bacteria: *E. coli* EBW25113 was grown at 37 °C in Luria Bertani (LB) broth or LB agar plates. 10% strength of LB broth was used for biofilm development.

Bulk Solution Electrochemical Experiments:

All electrochemical experiments were performed using a CH Instrument 1206B potentiostat. CVs were acquired in 5 mL of stationary solution in a three-electrode arrangement with a disk working electrode, Pt coil counter electrode and 3M Ag/AgCl reference electrode.

Bulk electrolysis with different applied potentials were performed in a cell containing 5 mL solution of 2 mM Cu(II)TPMA, 100 mM nitrite and 0.1 M MOPS buffer. The gas product was then swept into a nitric oxide analyzer (NOA Sievers 280i, GE). The schematic of experiment setup is shown below in Scheme 1.

Catheter Fabrication:

A single lumen silicone rubber tubing (7.5 cm length, inner diameter 1.47 mm, and outer diameter 1.96 mm) was sealed at one end with silicone rubber sealant. A polytetrafluoroethylene (PTFE)-coated silver/silver chloride wire (with 0.080 cm² surface area exposed) was used as the reference electrode, and a PTFE-coated platinum wire (with 0.039 cm² surface area exposed) was used as the working electrode. The exposed ends of the respective wires were coiled separately. The coiled ends were inserted into the single lumen silicone rubber tubing so that the silver/silver chloride wire and the copper wire were not in direct metallic connection. A solution was loaded into the single lumen silicone rubber tubing. The solution included 2 or 4 mM Cu(II)TPMA, 0.4 M NaNO₂, and 0.2 M NaCl in 0.5 M MOPS buffer (pH 7.2). The silicone rubber tubing was then sealed to form a catheter. The PTFE-coated silver/silver chloride wire and PTFE-coated Pt wire extended out of the silicone rubber tubing as respective leads to the reference and working electrodes. A dual lumen catheter may be formed in a similar manner by introducing the solution and electrode into one of the two available lumens.

Gas Phase IR Experiment:

A cell containing 10 mL of different concentrations of nitrite (50, 100 200 and 400 mM) in 2 mM CuTPMA, 0.2 M NaCl and 0.5 M MOPS buffer (pH 7.2) was used for bulk electrolysis. The solution was purged with Ar for 30 min before each experiment. A Pt wire electrode (0.32cm^2) was used as working electrode and Ag/AgCl wire as reference and counter electrode. A constant potential (-0.4 V vs. 0.2 M Ag/AgCl) was applied for 3 h with stirring, after which the headspace gas was transferred into a vacuumed gas phase IR cell using a cannula and analyzed using a Perkin-Elmer FT-IR. A prior calibration curve was obtained by adding different known amounts of saturated N_2O solution into 10 mL 0.5 M MOPS buffer (pH 7.2) to make 0.25, 1, 2 and 2.5 mM N_2O . Headspace N_2O was analyzed similarly and quantification was achieved by integration the N_2O feature peaks at 2235 and 2212 cm^{-1} in the IR spectra (see Table S-1 and Figure S-2).¹

Rabbit Experiments:

Animal handling and surgical procedures were approved by the University Committee on the Use and Care of Animals in accordance with university and federal regulations and the detailed protocols are reported elsewhere.² Briefly, two catheters were implanted into the left and right jugular veins of ca. 3 kg anesthetized rabbits. The animals received no other anticoagulant or anti-platelet agent during the experiment. One of the catheters was “turned on” by applying a -0.4V vs. 0.2 M Cl⁻ Ag/AgCl reference and the other “turned off” with no potential applied. After 7 h, the catheters were explanted and a digital picture was taken and the red pixels were counted using Image J software to represent the clot area.

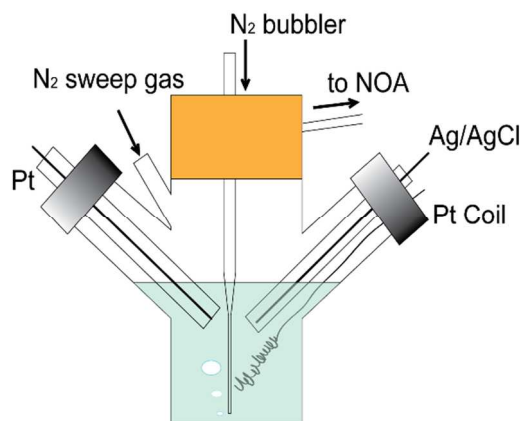
Numeric Simulation:

Simulation was performed using COMSOL 4.3 b. To determine the NO distribution around the dual lumen catheter, a 2D space simulation was performed using the cross sectional geometry of the catheter. Since the catheter has large length to diameter ratio, the edge effect of the two ends of the catheter can be neglected.

For NO distribution along the single lumen catheter, 2D-axial symmetry space was used. An NO flux of $7.4 \times 10^{-9} \text{ mol min}^{-1} \text{ cm}^{-2}$ was set from the electrode surface in both cases, which is calculated based on NO release experimental data from the NOA. A diffusion profile with no convection is set with the stiff spring boundary condition across the solution-polymer phase boundary, which is used to ensure a continuous flux with partitioning of NO between the two phases (solution and polymer). Key boundary conditions and parameters are listed in Table S-3. The diffusion and partition coefficients are based on literature data.³⁻⁵

ICP-OES Experiment:

NO releasing single lumen catheters were turned on (-0.4V) and soaked in 3 mL PBS buffer (pH 7.4) for 7 days (n=3). The Cu content in both soaking PBS solution and the native PBS solution was detected by ICP-OES and quantified via a calibration curve method. The Cu content results are summarized in Table S-4.



Scheme 1. Experiment setup for bulk electrolysis of Cu(II)TPMA and nitrite solution

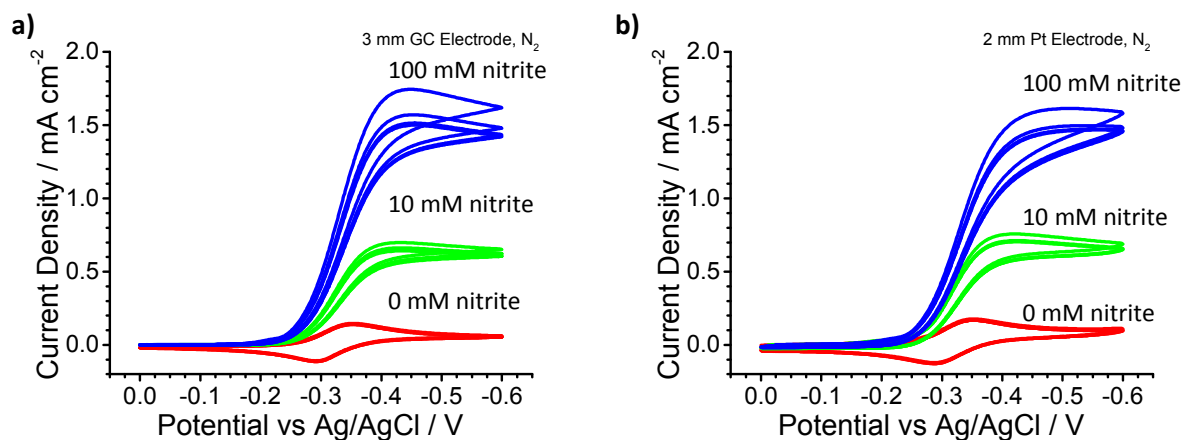


Figure S-1. Cyclic voltammogram of 1 mM CuTPMA in 0.1 M MOPS buffer (pH 7.2) in a bulk solution experiment using a) 0.0707 cm^2 glassy carbon disk electrode; and b) 0.0314 cm^2 platinum disc electrode with different levels of nitrite in N_2 environment. Scan rate = 50 mV/s.

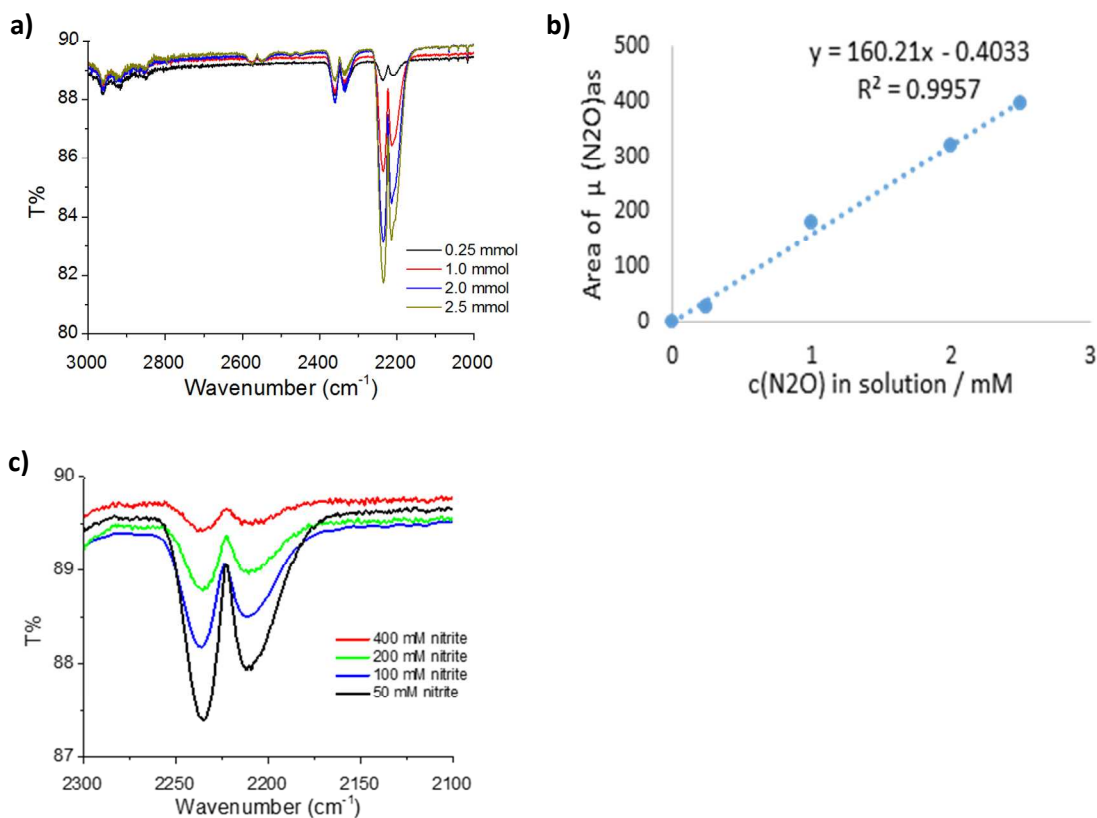


Figure S-2. a) Gas phase IR spectra of headspace N_2O solution standards; b) calibration curve from integration of N_2O feature peaks at 2235 and 2212 cm^{-1} ; c) gas-phase N_2O produced from bulk electrolysis of CuTPMA with different levels of nitrite present in the solution phase.

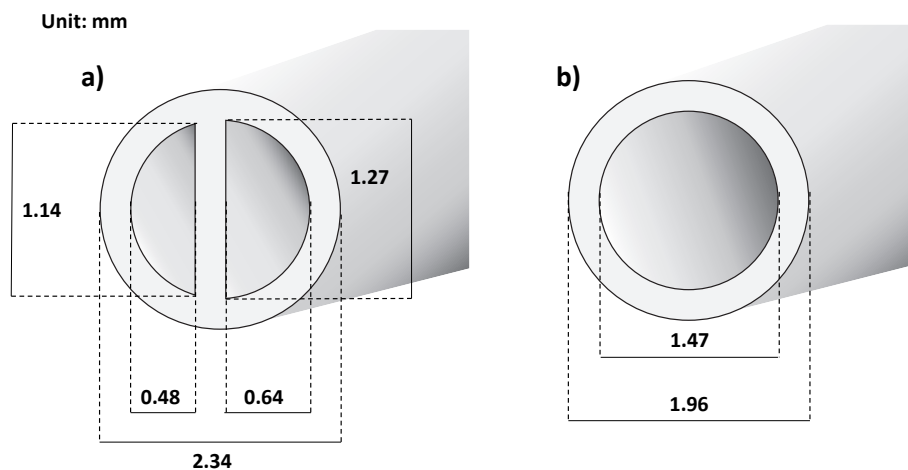
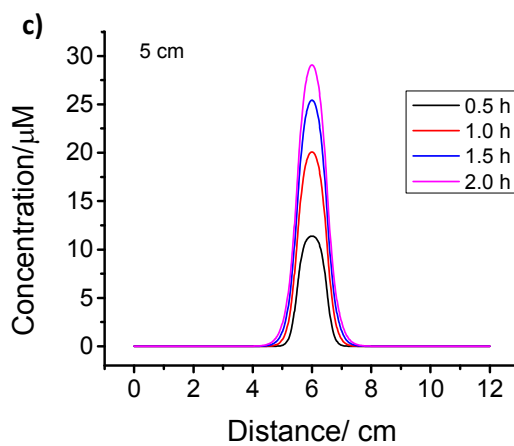
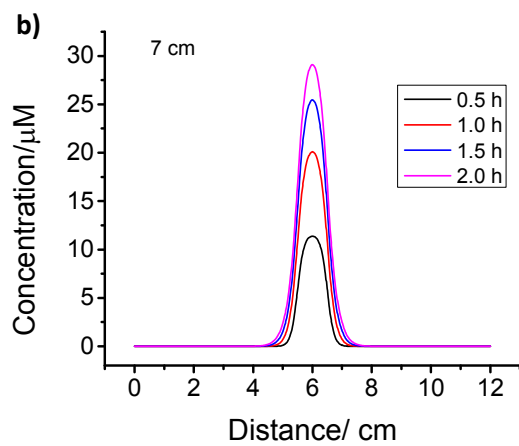
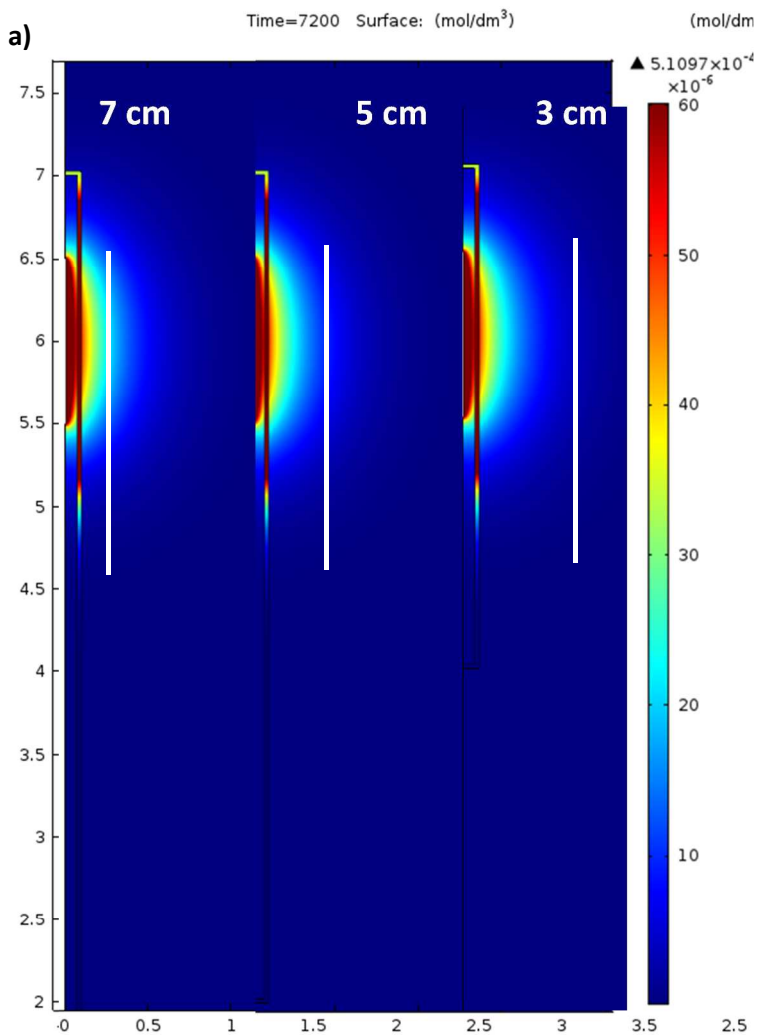


Figure S-3. Cross sectional geometries of a) dual lumen and b) single lumen silicone catheters employed in these studies.



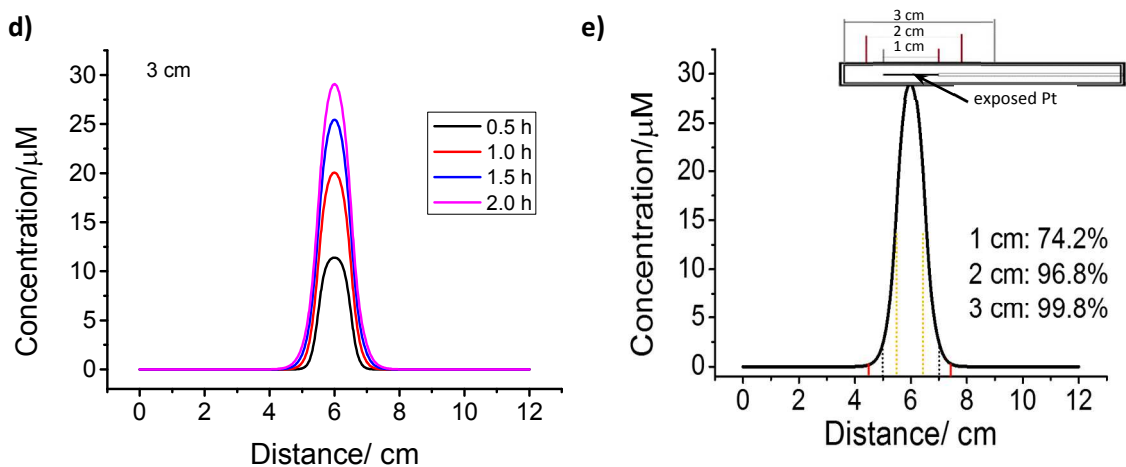


Figure S-4. a) The concentration profile of 7, 5 and 3 cm catheters with 1 cm long active Pt surface; concentration profile 100 μm away from catheter surfaces of b) 7 cm c) 5 cm d) 3 cm in length along the direction of the white line at different time points; e) integration of concentration of a 7 cm catheter, with 1, 2 and 3 cm along a line 100 μm away from catheter surface near the exposed Pt electrode.

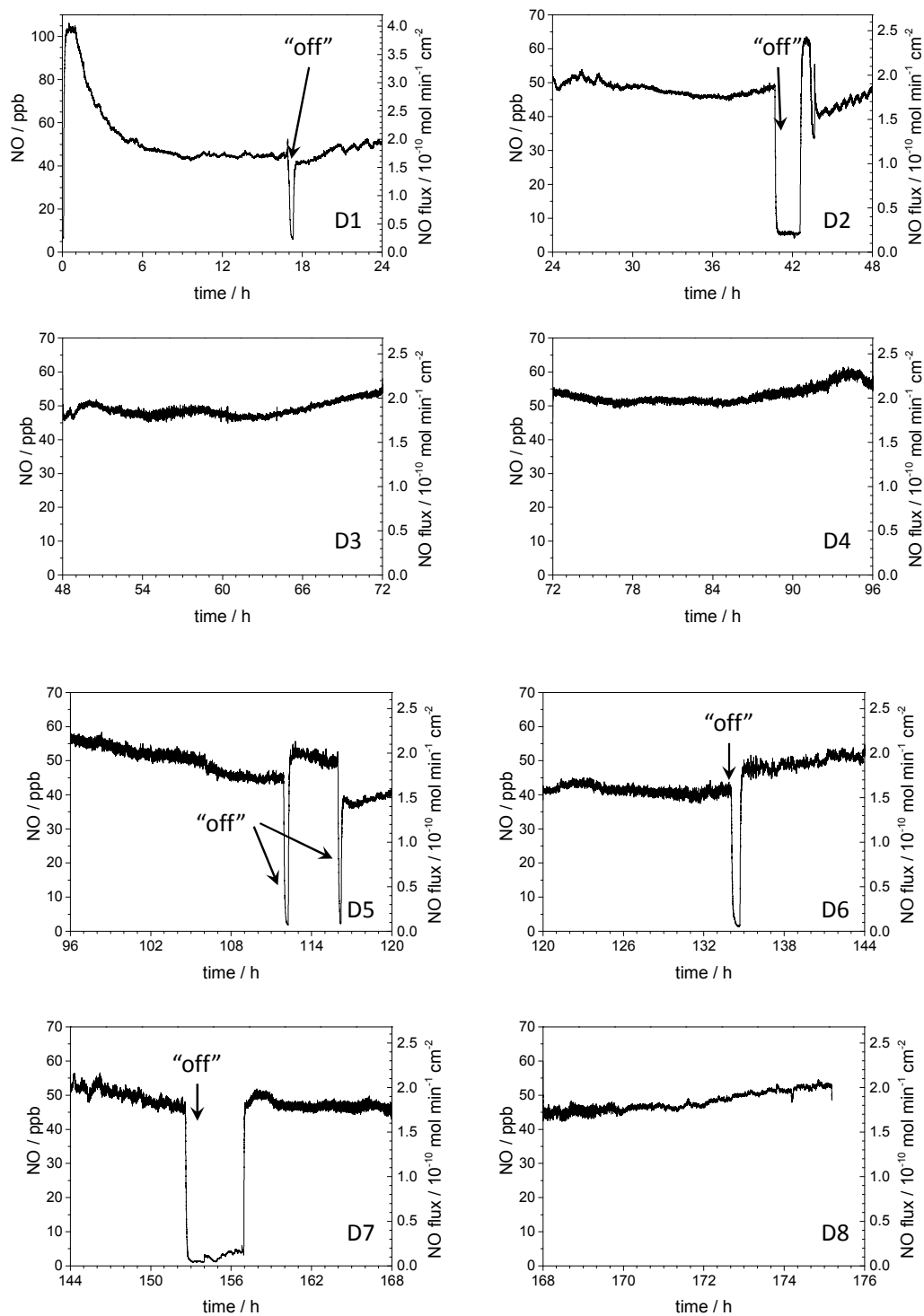
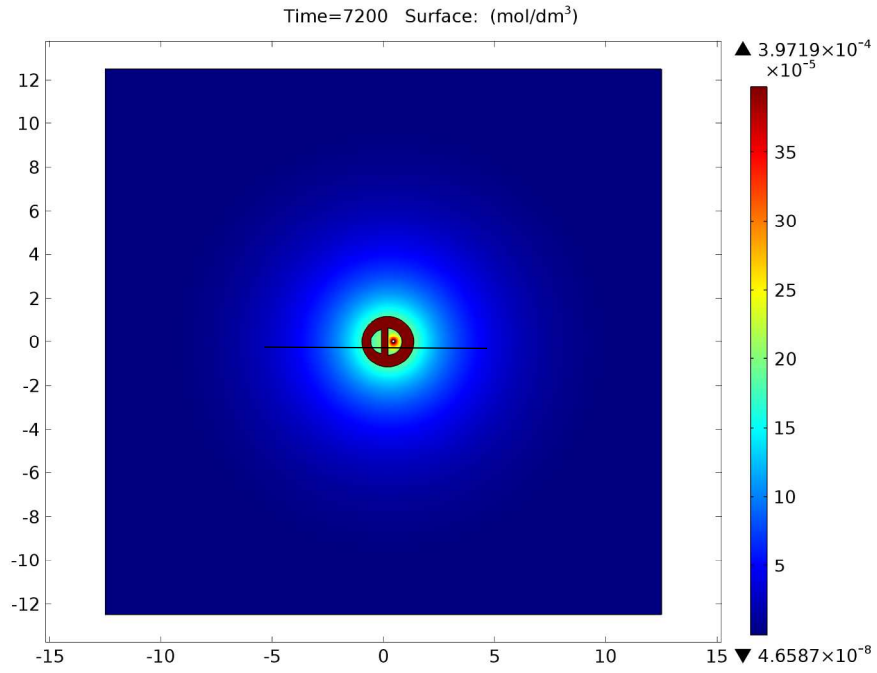


Figure S-5. Nitric oxide release from a 7.5 cm long single lumen catheter with 0.080 cm^2 Pt wire. Inner solution contains 2 mM Cu(II)TPMA, 0.4 M NaNO_2 , 0.2 M NaCl and 0.5 M MOPS (pH 7.2). NO turned "on" (-0.4V vs. 0.2 M Cl⁻ Ag/AgCl reference) for 8 d; periods of applied potential being turned "off" are also indicated.

a)



b)

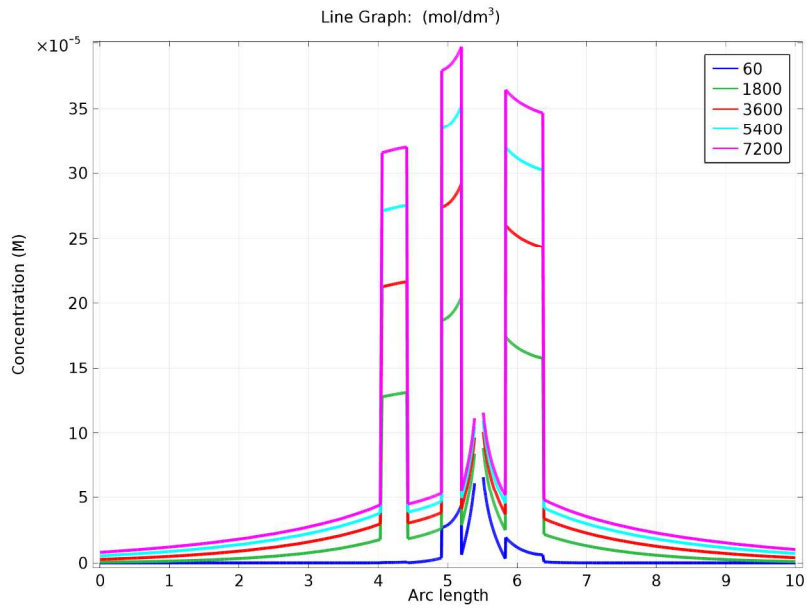


Figure S-6. Simulation of NO diffusion through dual lumen silicone catheter (via COMOSOL Multiphysics). a) NO concentration profile; b) cross line (black line in a)) concentration profile.

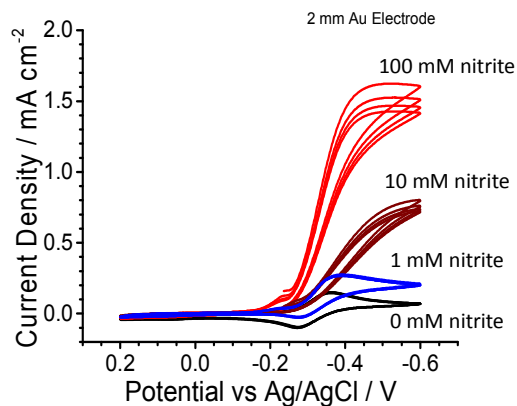


Figure S-7. Cyclic voltammogram of 1 mM Cu(II)TPMA in 0.1 M MOPS buffer (pH 7.2) in the bulk solution with a 0.0314 cm² gold disc working electrode with different levels of nitrite. Scan rate is 50 mV/s.

Table S-1. The effect of nitrite concentration on the current efficiency and gaseous product ratio between NO and N₂O using CuTPMA mediated nitrite electrochemical reduction.

c(NO₂⁻)/ mM	Area of μ(N₂O)_{as}	n(N₂O)/ μmol	N₂O current efficiency	NO : N₂O
50	91	2.9	68%	1 : 3.1
100	54	1.7	41%	1 : 0.5
200	28	0.9	21%	1 : 0.3
400	12	0.4	9%	1 : 0.06

Table S-2. Potential applied, NO flux, and NO current efficiency in the single lumen catheter.

Potential[*]/V	NO flux/ 10⁻¹⁰ mol min⁻¹ cm⁻²	NO current efficiency
-0.20	0.4	81%
-0.25	1.5	80%
-0.28	2.5	59%
-0.30	2.9	43%
-0.32	3.0	31%
-0.40	3.0	10%

* Potentials are vs. 0.2 M Ag/AgCl

Table S-3. Boundary conditions used for simulating the NO diffusion from electrode surface through silicone rubber.

Boundary/ domain	Boundary Conditions
Electrode surface	$N_1 = 7.4 \times 10^{-9} \text{ mol min}^{-1} \text{ cm}^{-2}$
Inner solution	D_{NO_water}
Inner solution-silicone	$N_2 = 1000 \times (K * c_1 - c_2)$
Silicone	$D_{NO_silicone}$
Silicone-outer solution	$N_3 = 1000 \times (c_2 - K * c_3)$
Outer solution	D_{NO_water}

N_1 is the flux of NO from electrode surface. N_2 is the flux of NO from the inner solution (Cu(II)TPMA and nitrite solution) into the silicone rubber. N_3 is the flux of NO from silicone rubber into the outer solution phase. D_{NO_water} and $D_{NO_silicone}$ are the diffusion coefficient of NO in water and silicone rubber, respectively. K is the diffusion coefficient of NO in silicone phase over water phase.

Table S-4. Result of 7-day Cu leaching experiment determined by ICP-OES

	Intensity	Concentration / ppb
Native PBS	3675 ± 263	10 ± 4
7 d Soaking PBS	3591 ± 293	10 ± 4

References:

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3. Zacharia, I.; Deen, W. Diffusivity and Solubility of Nitric Oxide in Water and Saline. *Ann. Biomed. Eng.* **2005**, *33*, 214–222
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5. Mowery, K. A.; Meyerhoff, M. E. The Transport of Nitric Oxide through Various Polymeric Matrices. *Polymer* **1999**, *40*, 6203–6207.