Supporting Information

Reduction of Thrombosis and Bacterial Infection via Controlled Nitric Oxide (NO) Release from S-Nitroso-N-acetylpenicilamine (SNAP) Impregnated CarboSil Intravascular Catheters

Yaqi Wo a, Elizabeth J. Brisbois b, Jianfeng Wu c, Zi Li a, Terry C. Major b, Azmath Mohammed b, Xianglong Wang d, Alessandro Colletta a, Joseph L. Bull d, Adam J. Matzger a, Chuanwu Xi c, Robert H. Bartlett b, Mark E. Meyerhoff a.*

aDepartment of Chemistry, bDepartment of Surgery, University of Michigan Medical Center, and cDepartment of Environmental Health Sciences, University of Michigan, Ann Arbor, MI 48109, USA

*Corresponding Author

Dr. Mark E. Meyerhoff
930 N. University Ave.
Ann Arbor, MI 48109
Telephone: (734) 763-5916
E-mail: mmeyerho@umich.edu
1. **SNAP synthesis protocol**

SNAP was synthesized as previously reported.\(^1\)\(^-\)\(^3\) In brief, an equimolar ratio of NAP and NaNO\(_2\) was added to a 1:3 mixture of water and methanol containing 2 M H\(_2\)SO\(_4\) and 2 M HCl. The reaction vessel was placed in an ice bath for 5 h while the green SNAP crystals precipitated. The crystals were collected by vacuum filtration, rinsed with iced DI water to remove any residues and allowed to air dry for 24 h before being stored in the freezer at -20 °C. The entire synthesis process was performed in the absence of ambient light.

2. **Polarized microscope images**

SNAP-impregnated polymer films with 5 wt% SNAP loading and a blank reference film of CarboSil, both without topcoats, were prepared as described above. Optical images were captured by a Leica DM2500 LED Microscope (from Leica Microsystems 2016) with a 20× and a 50× objective under crossed polarizers. The images were taken using the Surveyor Software, specifically the 20 x objective image was taken by mosaic imaging and the 50 x objective image (insert) was taken by extended depth of field (EDF) imaging. To better observe the crystal distribution within the impregnated CarboSil polymer, very thin slices (30 µm in thickness) of cross-cross-sections of the 5 wt% SNAP-impregnated CarboSil slices were prepared and observed under the microscope. The film was first cut to expose its cross-section and then embedded into optimal cutting temperature compound (OCT), allowed to harden at -20 °C, and then cut carefully into 30 µm thick slices by the Leica CM3050S research cryostat. The polymer film slices were then observed under the microscope plate for crystal distribution.

3. **Powder X-ray diffraction measurements**
SNAP-impregnated polymer films with various wt% SNAP loading and a blank reference film of corresponding polymer, all without topcoats, were prepared as described in Section 2.2. Powder X-ray diffraction (PXRD) 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 = 1.54187Å) at 40 kV and 44 mA. Synthesized SNAP crystals were finely ground to eliminate preferred orientation, whereas blank CarboSil and SNAP-impregnated CarboSil samples were cut into cubes with dimensions of approximately 250 µm. 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°/sec, the φ-axis was rotated at 10°/sec, and χ-axis was fixed at 45°. The obtained images were integrated from 2.5 to 70° with a 0.1° step size in AreaMax 2.0 software from Rigaku. All powder patterns were processed using Jade 9 XRD Pattern Processing, Identification & Quantification analysis software from Materials Data, Inc. The simulated powder patterns of monoclinic and orthorhombic SNAP crystals were calculated in Mercury 3.3 from the CCDC and were compared with the experimental SNAP powder pattern in Jade 9. Quantitation of peak intensity ratios versus SNAP weight percentage was performed in KaleidaGraph 3.5.

4. Effects of ethylene oxide (EO) sterilization

SNAP-impregnated CarboSil films (15 wt%) were prepared and sent to the University of Michigan hospital sterilization facility for ethylene oxide (EO) treatment, a standardized procedure for many medical devices used in clinical applications. Briefly, the films went through 1 h of preconditioning and humidification process, followed by 2-3 h of EtO gas exposure, both performed in a high temperature (54 °C) and high humidity environment (40-80
After the 1-2 h of EtO gas evacuation (54 °C), the films were subjected to 12 h of air washes (54 °C). The amount of SNAP remaining in the films was determined by UV-Vis absorbance measurements as previously reported.

5. In vitro evaluation of antibiofilm properties of SNAP-impregnated CarboSil catheters in CDC biofilm reactor

A CDC biofilm reactor system was used to test the anti-biofilm properties of SNAP/CarboSil catheters against biofilm formed by *S. epidermidis* and *P. aeruginosa*. The CDC biofilm reactor, its coupon holders and all other connection tubing were autoclaved before use. The EO sterilized catheters were first mounted onto the coupon holders within the reactor and the reactor was then supplemented with sterile nutrient medium (10% Luria Broth) inoculated with overnight bacteria culture (1 to 100 dilution, ~1 × 10^6 CFU/mL) for 1 h to allow bacterial cell adhesion on the catheter surfaces. The continuous flow of 10 wt% LB broth was controlled at the flow rate of 100 mL/h by a peristaltic pump for 14 days, at 37 °C. The liquid medium was circulated through the vessel and a magnetic stir bar rotated by a magnetic stir plate was used to generate shear force. After incubation for 14 d, the catheters were aseptically removed and each catheter was cut into two 1 cm pieces, that were used for plate counting and imaging, respectively. For plate counting, the catheter segment was homogenized (OMNI TH, Omni International, Kennesaw, GA) for 30 s in 2 mL of 10 mM sterile PBS (pH 7.4) in order to disintegrate the biofilm to a suspension of isolated cells, which was then diluted by 10-fold each time and plated onto LB agar plates. For imaging, the catheter segment was stained with Live/Dead BacLight Bacterial Viability kit (Invitrogen, Carlsbad, CA) for 20 min in the dark, exactly per the kit’s instructions. Fluorescence images were acquired with an inverted fluorescence microscope (Olympus IX71,
Center Valley, PA) equipped with Fluorescence Illumination System (X-Cite 120, EXFO) and filters for SYTO-9 (excitation = 488 nm/emission = 520 nm) and Propidium Iodide (excitation = 535 nm/emission = 617 nm). Images were obtained using an oil immersed 60× objective lens, in which red indicates dead and green indicates live bacteria.

6. In vivo evaluation of SNAP-impregnated CarboSil catheters in rabbits

A total of 3 New Zealand white rabbits (Covance, Battle Creek, MI) were used in this study. All rabbits (2.5-3.0 kg) were initially anesthetized with intramuscular injections of 5 mg/kg xylazine injectable (AnaSed® Lloyd Laboratories Shenandoah, Iowa) and 30 mg/kg ketamine hydrochloride (Hospira, Inc. Lake Forest, IL). Maintenance anesthesia was administered via isoflurane gas inhalation at rate of 1.5-3% by mechanical ventilation via a tracheotomy and using an A.D.S 2000 Ventilator (Engler Engineering Corp. Hialeah, FL). Peek inspiratory pressure was set to 15 cm of H₂O) and the ventilator flow rate was 8 L/min. To facilitate the maintenance of blood pressure stability, IV fluids of Lactated Ringer’s were given at a rate of 10 mL/kg/h. In order to monitor blood pressure and to collect intermittent blood samples for analysis during the experiment, the rabbit’s right carotid artery was cannulated using a 16-gauge IV angiocatheter (Jelco®, Johnson & Johnson, Cincinnati, OH). The blood pressure and derived heart rate were monitored by a Series 7000 monitor (Marquette Electronics Milwaukee, WI) while the animal body temperature was monitored with a rectal probe and maintained at 40 °C using a water-jacketed heating blanket. Sample blood analysis (arterial blood pH, PCO₂, PO₂, total hemoglobin and methemoglobin) was conducted using an ABL 825 blood-gas analyzer and an OSM3 Hemoximeter (Radiometer Copenhagen, DK). Prior to the placement of catheters, the rabbit left and right external jugular veins were isolated. Five cm lengths of the catheters (one
SNAP and one control) were inserted into the veins. The animals were not treated with anticoagulant systemically during the experiments.

During the experiment, the mean arterial pressure (MAP) of the rabbit was maintained at 35 ± 5 mmHg for 7 h by continuous IV fluid maintenance. The heart rate average was 225 ± 10 beats/min and no significant change was noted for the duration of experiments. The blood gas was measured once every hour and the results were all within the normal ranges. Rabbit whole blood samples were collected in non-anticoagulated 1 cc syringes for activated clotting times (ACT) analysis at the beginning of the experiments. The whole blood samples were also collected by 1 cc heparinized syringes (40 U/mL of sodium heparin) for blood gas analysis every hour for 7 h.

After 7 h of catheter implantation, all animals were first given (400 U/kg) sodium heparin (APP Pharmaceuticals, LLC Schaumburg, IL) systemically to prevent thrombosis during necropsy and were then euthanized with a dose of Fatal Plus (130 mg/kg sodium pentobarbital) (Vortech Pharmaceuticals, Dearborn, MI). The jugular veins were clamped and the catheters were carefully removed from the vein, leaving the thrombus intact on the catheter surface. After rinsing the catheter with saline, any residual thrombus was photographed and quantitated using Image J imaging software provided by the National Institutes of Health (Bethesda, MD).
Figure S1. The schematic process of SNAP impregnation into CarboSil polymeric material using a CarboSil catheter as an example.

Figure S2. The wt % SNAP impregnated in the final CarboSil polymer films correlated with the SNAP concentration in swelling solution consisting of 70% MEK and 30% MeOH and various concentrations of SNAP.
Figure S3. Optical image of A) blank CarboSil film and B) 5 wt% SNAP-impregnated CarboSil film surfaces taken under crossed polarizers in combination with a quarter-wave plate. The 5 wt% films present patterns which suggest the presence of crystalline SNAP in the polymer.
Figure S4. Schematic diagram of SNAP distribution in CarboSil polymer prepared by A) casting SNAP and CarboSil polymer solution; and B) polymer solvent impregnation. The lines in the diagram correspond to CarboSil polymer chains, the diamonds correspond to SNAP molecules, and the dots correspond to solvent used in the preparation.
Reference:

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