Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information

Nitric Oxide-Releasing Semi-Crystalline Thermoplastic Polymers: Preparation, Characterization and Application to Devise Anti-Inflammatory and Bactericidal Implants

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Materials and Reagents

Pebax 6333 tubing (I.D.: 0.016'', O.D.: 0.036''), Pebax 7033 tubing (I.D.: 0.020'', O.D.: 0.040''), Pebax 7233 tubing (I.D.: 0.018'', O.D.: 0.038''; I.D.: 0.0134'', O.D.: 0.0228''(24 gauge); I.D.: 0.018'', O.D.: 0.034'' (20 gauge)), Nylon (I.D.: 0.028'', O.D.: 0.048''), Pellethane 55D (I.D.: 0.070'', O.D.: 0.078''), and Pellethane 75D (I.D.: 0.040'', O.D.: 0.048'') were purchased from Vention MEDICAL (now as Nordson MEDICAL). SNAP was purchased from Pharmablock (USA) Inc. Anhydrous dichloromethane, anhydrous methanol, and all other chemicals were purchased from Millipore-Sigma.

Impregnation of Biomedical Tubes with SNAP

Two hundred fifty mg/mL SNAP was dissolved in a mixed solvent of anhydrous dichloromethane (70 vol%) and methanol (30 vol%) at 37 °C. Decomposition of this SNAP solution over 3 h at 37 °C was measured to be 4.6 ± 1.7 % (n=3) based on its absorbance at 596 nm (10 times dilution in methanol was employed for the spectrophotometry). PEBA and Nylon 12 tubes were soaked in this solution for 3 h in the dark at the same temperature. After being removed from the solution, the tubes were left in air for 2 h and then were dried in vacuum for 12 h to allow evaporation of solvents. All tubes were rinsed extensively with methanol to remove residual SNAP on both the inner and outer tube surfaces and were then dried again. Impregnation of Pellethane with SNAP uses the same protocol except that a lower ratio of dichloromethane (50 vol%) was employed. All tubes were stored in the dark at -20 °C when not in use.

Test of NO Release as Well as Loading and Leaching of SNAP

NOA was used to test the NO release of various tubes in PBSE in a homemade NOA cell at 37 °C. A gas mass flow controller (Alicat Scientific) was used to control the flow rate of the inlet gas to 200 SCCM. Ten tubes of 2 cm length were tested in the same cell and the reported data is their averaged NO flux. Cannula tubes were always soaked in PBSE at 37 °C when they are not being tested. To determine

the loading of SNAP in PEBA/Nylon 12 tubes, the binary solvent optimized for impregnation was used to extract SNAP from the tubing at 37 °C. Extractions were performed three times for each set of chopped tubes to transfer all SNAP from the polymer to the solvent. The amount of SNAP in the extraction solvent was determined by injection of a 50 μL aliquot of sample into a NOA cell containing 1 mM $CuCl_2$ and 0.4 mM L-cysteine in DI water. The amount of NO released over *ca* 0.5 h period was integrated to determine the total SNAP present in the tubes. Cumulative leaching of SNAP, NAP, and NAP dimer from the SNAP-doped tubing into PBSE was determined by liquid chromatography−tandem mass spectrometry (6520 Accurate-Mass QTOF LC/MS, Agilent Technologies) using a reversed-phase column (ZORBAX RRHD Eclipse Plus C18, 2.1 x 50 mm, 1.8 μm). For chemical leaching of each type of SNAP-doped polymer tubes, the data is from eight 1 cm long tubes and the soaking solution is 500 uL of PBSE. All cannulas were placed in fresh PBSE immediately after each measurement while the previous soaking solution was analyzed. The elution gradient was obtained with eluent A (water with 0.1% formic acid) and eluent B (acetonitrile with 0.1% formic acid). After sample injection (5 μL), 95% eluent A was flowed for 5 min and a linear change of eluent mixtures from 95% A to 0% A over 10 min was carried out afterwards at a flow rate of 0.4 mL/min. The amount of total chemical leaching was calculated as follows: [total chemical] = [SNAP] + [NAP] + 2 x [NAP disulfide].

Characterization of Polymer Tubes

All solid-state NMR data was acquired using a 400 MHz Bruker AVIII spectrometer, equipped with a 4 mm two- channel MAS probe. Four tubes of ~2 cm length were placed into a clean, dry 4 mm Zircon rotor and sealed with Kel-F drive cap. For test of SNAP powder, Al_2O_3 was added as an inert filler to distribute mass evenly across the rotor and facilitate spinning. WAXD spectra were collected at room temperature using a Rigaku R-Axis Spider diffractometer equipped with an image plate detector and graphite monochromated Cu−Kα radiation (γ=0.154 nm). The tube was mounted on a modeling clay and

the X-ray was along the longitudinal direction of the tube. AFM was performed on a Veeco Dimension Icon Atomic Force Microscope in PeakForce QNM mode and a ScanAsyst-Air probe was used. Tensile tests were performed on an Admet eXpert 8600 machine. The tubes were pulled apart at a constant rate of 50 mm/min (strain rate of 0.107 s^{-1}) until failure. Tensile strength, tensile strain, and Young's modulus were extracted from the raw data using MATLAB 2014a from MathWorks. Statistical analysis on these parameters was performed in PSPP 1.0 of the GNU Project. DSC and TGA were performed under a nitrogen atmosphere by using a Perkin-Elmer DSC-7 Differential Scanning Calorimeter and TGA-7 Thermogravimetric Analyzer with a heating rate of 20 °C/min. Photos of tubes was taken using a homemade black box and an iPhone. Size of the different tubing was normalized in all photos.

***In Vitro* Biofilm Test**

S. epidermidis and *S. aureus* strains were co-cultured with 24-gauge control and NO-release Pebax 7233 tubes at 37 °C for 3 and 7 days in a CDC reactor supplemented continuously with 10% strength of LB broth at a flow rate of 100 mL/h. Ethylene oxide sterilization was employed for all tubes before the biofilm experiment. After incubation, tubes were aseptically removed and were cut into two identical parts. One part was used for estimating the viable bacteria adhered on the outer surface by using plate counting method. Another part was stained with LIVE/DEAD BacLight Bacterial Viability kit (L7012, Thermofisher Sci.) before obtaining representative images on the surface by using an inverted fluorescence microscope with 60 × lens.

***In Vivo* Test of Pebax Tubing as Subcutaneous Insulin Infusion Cannula in a Sheep Model**

All protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Michigan, Ann Arbor. Veterinary care and histology services were provided by the Unit for Laboratory Animal Medicine (ULAM), University of Michigan, Ann Arbor. After 24 h of fasting and pre-procedural transdermal fentanyl administration (100mcg/hr), adult sheep (>45 kg) were sedated using

ketamine (6-12 mg/kg intramuscular) and diazepam (1 mg/kg intramuscular). A 9-French single-lumen catheter was placed in the right jugular vein using sterile Seldinger technique and used for blood draws and drug/glucose administration. Under conscious sedation (propofol 2 mg/kg intravenous every 5 min as needed), the skin of the back was carefully shaved and prepared with Chlorhexidine. Pebax 7233 tubes (24- or 20- gauge) with or without SNAP were introduced into the subcutaneous space on the back of the animals using appropriately sized 22- or 18-gauge needles at an insertion angle of ~30°. Each Pebax tube was 3 cm long with 1 cm outside of body for connection to the infusion tube and 2 cm under the skin. At least a 4 cm distance was preserved between the control cannula and the NO-releasing cannula. Two pairs of 24-gauge cannula and two pairs of 20-gauge cannula were implanted in 4 sheep (i.e., one animal has one pair of cannula for intravenous glucose tolerance test). Another two pairs of 20-gauge tubes were implanted in one sheep and the distance between any two cannulas was at least 4 cm.

Animals were recovered in a PVC stanchion and monitored continuously in chronic housing, where cannulas were connected to an infusion pump to deliver a low rate (10 uL/h) of 0.9% sodium chloride solution for catheter patency. This rate is equivalent to a typical basal rate infusion for commercial insulin pumps. Intravenous glucose tolerance tests were conducted once daily for 14 days for the animal with one pair of cannula, or twice daily (with 8 h apart) for 14 days for the animal with two pairs of cannula. For each test, a baseline blood glucose level was measured. Then an intravenous glucose challenge was administered (50% dextrose solution, 300 mg/kg intravenous push) and simultaneously an infusion of 15 units of insulin Lispro was administered over 20 min via a controlled rate syringe pump, through either the control or SNAP-doped subcutaneous cannula. Blood glucose samples were drawn at baseline and 5, 10, 20, 30, 45, 60, 90, and 120 minutes after glucose infusion. Intrinsic glucose tolerance was evaluated on day 15 with a glucose tolerance test without extrinsic insulin infusion (glucose injection only). On day 15 after the blank glucose tolerance test, animals were anesthetized with propofol (5 mg/kg)

and euthanized per IACUC guidelines. Tissue surrounding the cannula was collected with a 2 cm circumferential margin, extending deeply to the underlying muscle fascia. Samples were formalin fixed and submitted to ULAM for blinded histology review. The inflammation density was scored as 0 (none), 1 (minimal; few scattered inflammatory cells), 2 (mild; greater numbers of mixed inflammatory cells), 3 (numerous mixed inflammatory cells adjacent to catheter), 4 (dense sheets of mixed inflammatory cells dissecting through fascial planes with tissue remodeling) and the inflammation extent was also scored as 0 (none), 1 (minimal; extending < width of radius of the catheter), 2 (mild; extending to the width of the radius of the catheter from the catheter edge), 3 (moderate; extending the radius to diameter of the catheter from the edge), 4 (severe; extending > the width of the catheter from the edge).

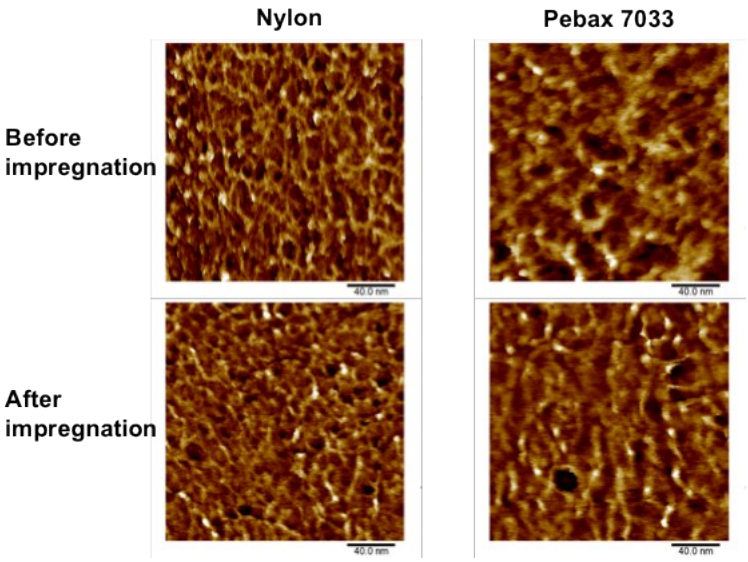

Figure S1. Distribution maps of adhesion over the outer surface of the tube. The image size is 0.2 μm*0.2 μm.

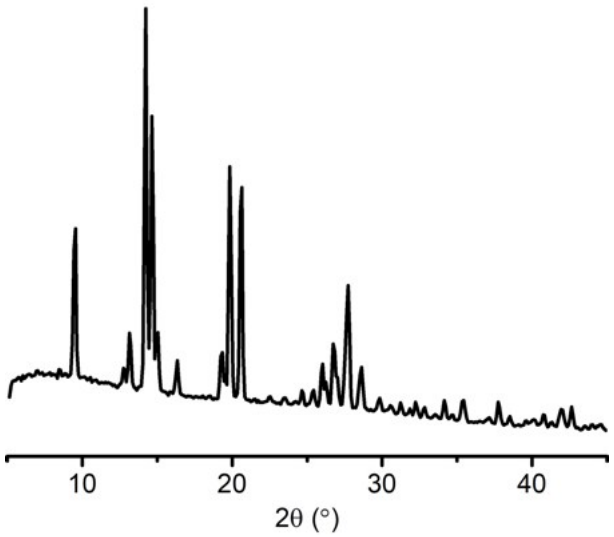

Figure S2. 1D WAXD pattern of SNAP powder under the same experimental conditions with those used for Figure 3.

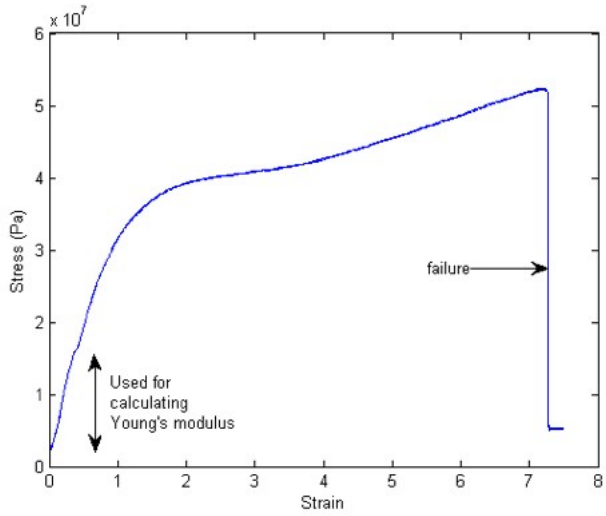

Figure S3. A representative stress-strain curve of a Nylon 12 sample undergoing tensile testing to failure. The stress and strain just before failure was recorded to be the ultimate tensile strength and strain (elongation) of the tube. The Young's modulus was calculated using the initial elastic segment as shown on the figure. The segment before the first visible "kink" was selected for all tubes to ensure consistency.

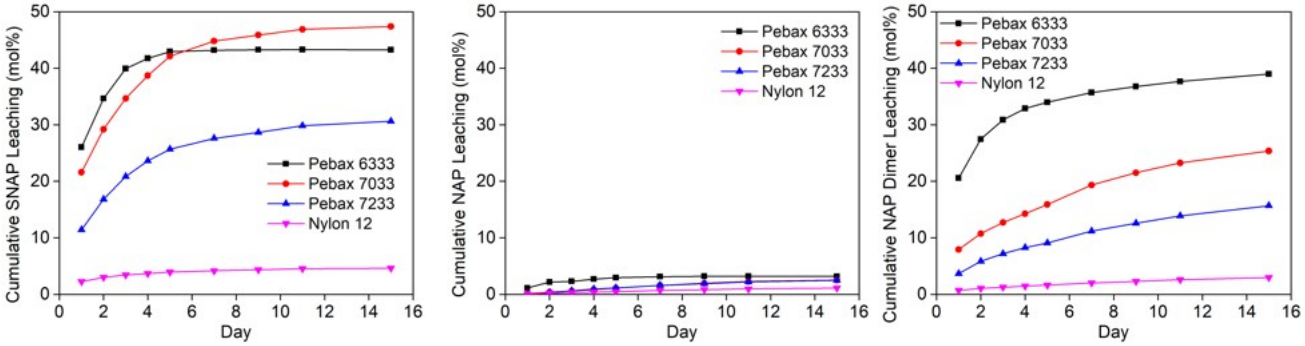

Figure S4. Cumulative SNAP, NAP, and NAP dimer detected in the soaking buffer. All molar percentages are relative to the original SNAP loading in the tubing before soaking. SNAP leaching was underestimated because SNAP is able to decompose into products in PBSE. Each leaching experiment used 8 tubes of 1 cm length.

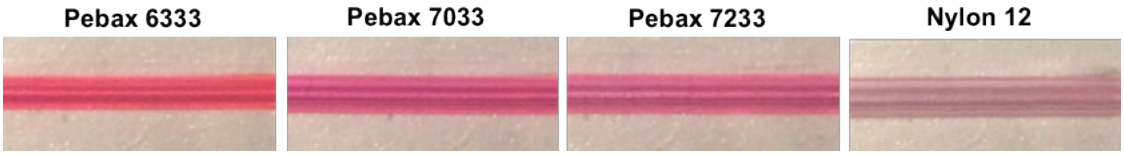

Figure S5. Color of four types of tubes after being soaked in aqueous solution of Rhodamine 6G for 24 h at 37 °C. This order is true for all dyes examined by us including methylene blue, methylene green, rhodamine 6G, brilliant blue G, sudan orange G, bromothymol blue, and potassium permanganate in DI water as well as alizarin in both DI water and 0.1 M HCl (data not shown). These dyes have various charged properties (cations, anions, zwitterion, neutral molecules), various sizes (158 to 854 in molecular weight), and various chemical natures (inorganic molecules and organic molecules with different functional groups). Therefore, their different degrees of penetration into these polymers are most likely due to the difference amount of unbound free water in the different polymers rather than specific interactions between a polymer and a dye.

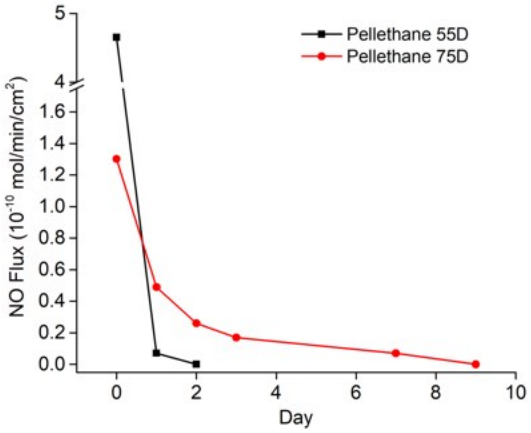

Figure S6. Nitric oxide release from four types of polymer tubes soaked in PBSE at 37 °C. Day 0 means NO release before pre-soaking. Five tubes of 2 cm length were tested in the same cell and the reported data point is their averaged NO flux.

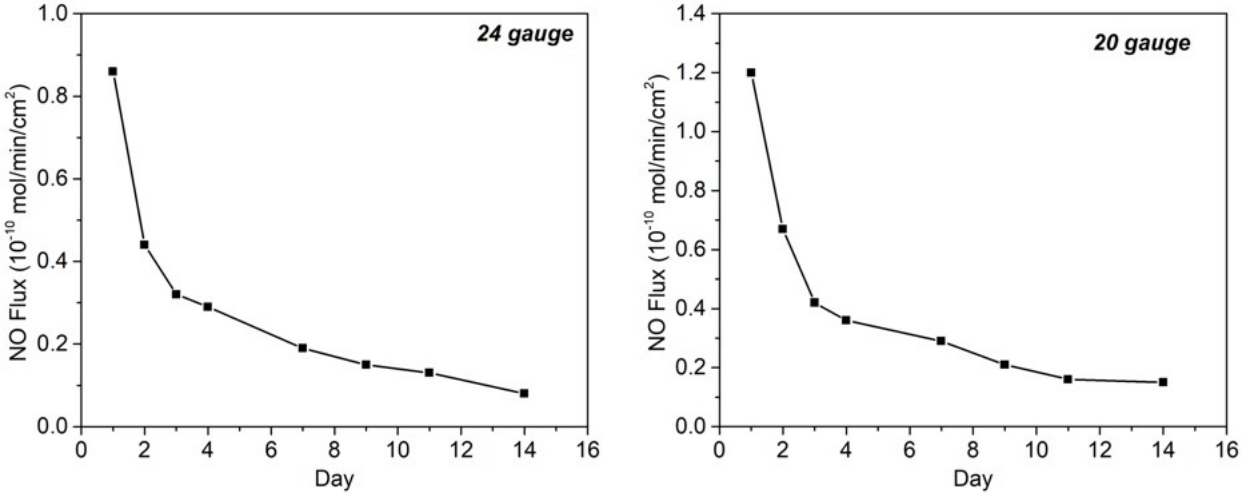

Figure S7. Nitric oxide release from differently sized Pebax 7233 tubes (24 gauge: I.D.: 0.0134'', O.D.: 0.0228''; 20 gauge: I.D.: 0.018'', O.D.: 0.034'') soaked in PBSE at 37 °C. Ten tubes of 2 cm length were tested in the same cell and the reported data point is their averaged NO flux.