Electrochemically Modulated Nitric Oxide Releasing Flexible Bactericidal Patch Prepared by 3-D Printing and Soft Lithography

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Supporting Information

Materials

Teflon PFA-coated platinum (0.125 mm OD) and silver (0.127 mm OD) wires were purchased from A-M Systems (Sequim, WA). Silver and stainless steel gauzes with 80 mesh was obtained from Alfa Aesar (Ward Hill, MA). Sylgard 184 silicone elastomer kit was prepared from Dow Corning Corporation (Midland, MI). Sodium chloride, sodium nitrite, coper acetate, tris (2-pyridylmethyl) amine, sodium bicarbonate, sodium carbonate, potassium chloride, and HEPES buffer were obtained from Sigma-Aldrich (St. Louis, MO). All chemicals was prepared in Mili-Q water (Millipore Corp., Billerica, MA). Luria Berani (LB) broth, LB agar, and aluminum foil roll were purchased from Fisher Scientific Inc. (Pittsburgh, PA). Compliant silicone rubber (NSF 51) was obtained from Rubber Sheet Roll (Shippensburg, PA) and Sil-Poxy Silicone Adhesive was from Smooth-On Inc. (Macungie PA). Polylactic acid plastic cartridge for 3D printer was prepared from 3D Systems Inc. (Rock Hill, SC).

Escherichia coli K-12 MG 16653 (*E. coli*) and *Staphylococcus aureus* ATCC 45330 (*S. aureus*) were obtained from the American Type Culture Collection (Manassas, VA).

Instrumentations

A portable potentiostat (CHI 1206B) from CH Instruments Inc. (Austin, TX) was used for the electrochemical experiments. The portable battery circuit system was prepared by Dr. Ondrej Novak and Dr. Richard Brown at the University of Utah). Nitric oxide release from wound healing patch system was measured with a nitric oxide analyzer from GE Instruments (Boulder, CO). Various dimensions of PDMS mold was fabricated with Cubify Cube 3D Printer 2nd Generation from 3D Systems Inc. (Rock Hill, SC).

Patch fabrication

The PLA mold of PDMS patch body was fabricated with 3D printer in UM3D Lab at the University of Michigan Ann Arbor. Patch body was prepared with 2 step curing processes of PDMS in order to embed an aluminum layer in the middle of a wall. Silgard 184 silicone elastomer base and curing agent was mixed at 10:1 ratio and transferred into PLA mold. It was cured at 100^{o}C for 40 min and cut with blade to yield a 1 – 2 mm thin layer. Then, it was wrapped with aluminum foil followed by addition of 2nd PDMS solution. After cured, a wall of PDMS body was cut to have 3 – 5 mm thick wall in Figure 1. PDMS spacer was also fabricated in PLA mold to prevent direct contact between working and reference/counter electrodes in a body structure. An Ag/AgCl gauze connected with Teflon PFA-coated silver wire was prepared with 9 × 27 or 24 × 72 mm (before folded) as a reference electrode. Gold coated stainless steel gauze was prepared by sputter coat of the stainless steel gauze with 50 nm of gold, and the gauze was connected with Teflon PFA-coated stainless steel wire was cut with 9 × 9 or 24 × 24 mm as a working electrode.

Ag/AgCl reference electrode, the spacer and the working electrode were sequentially inserted into PDMS body. Finally, they were covered with silicone rubber as a membrane, and sealed and cured with a silicone rubber sealant for 12 hours. Patch body was filled up with sodium nitrite (0.4 M) and CuTPMA (4 mM) catalyst via 27G stainless steel needle and sealed with a silicone rubber sealant in Figure 1.

In vitro antimicrobial experiment in solution

LB broth was inoculated with *E. coli* or *S. aureus,* and the bacteria were grown in the media at 37 °C for overnight culture. The overnight culture media was then diluted in 10 mM phosphate-buffered saline to a final concentration of 10^5 CFU/mL. A 10 ml aliquot of this diluted bacteria solution was transferred to a 50 ml of corning tube and a PDMS patch (40 × 40 × 6 mm, L × W × H) was immersed in bacteria solution. Portable battery power circuit with -0.44 V was connected to reference and working electrodes in wound patch system and incubated at 37^{o}C with 180 rpm of shaking. 1 ml of bacteria solution was spread on agar media after 6 hours and 24 hours for plate counting.

In vitro antimicrobial experiment on surface

One mL of *E. coli* or *S. aureus* culture in LB broth media (10^5 CFU/mL) was spread on agar plates. Two PDMS patches (40 x 40 mm, L x W) were placed on an agar plate of each bacterial strain with silicone membrane touching the agar plate directly. A sample patch was connected to the battery power circuit, and a control one was not connected to any power system. Both patches on agars were cultured at 37^{o}C for overnight and gently removed. The agar plates without patches were cultured at 37^{o}C for another overnight for plate counting.

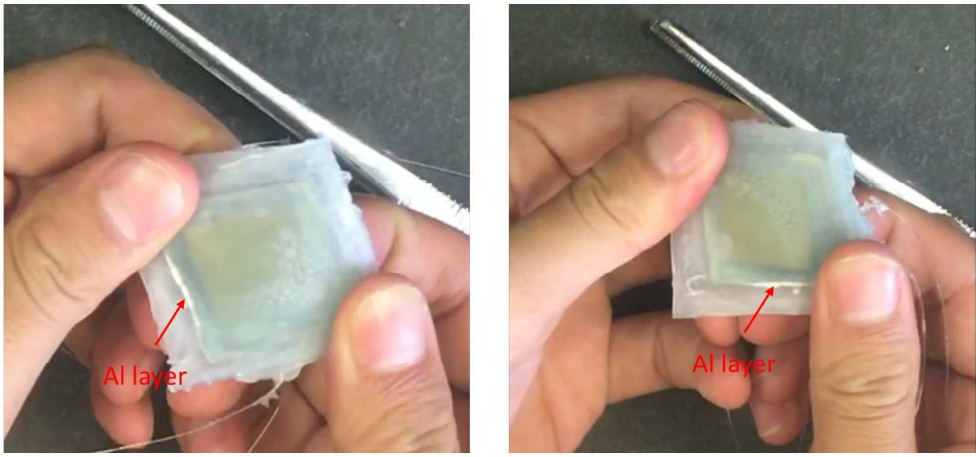

Figure S1. Bending of the NO releasing patches showing its flexibility. Aluminum layer is indicated by the arrow.

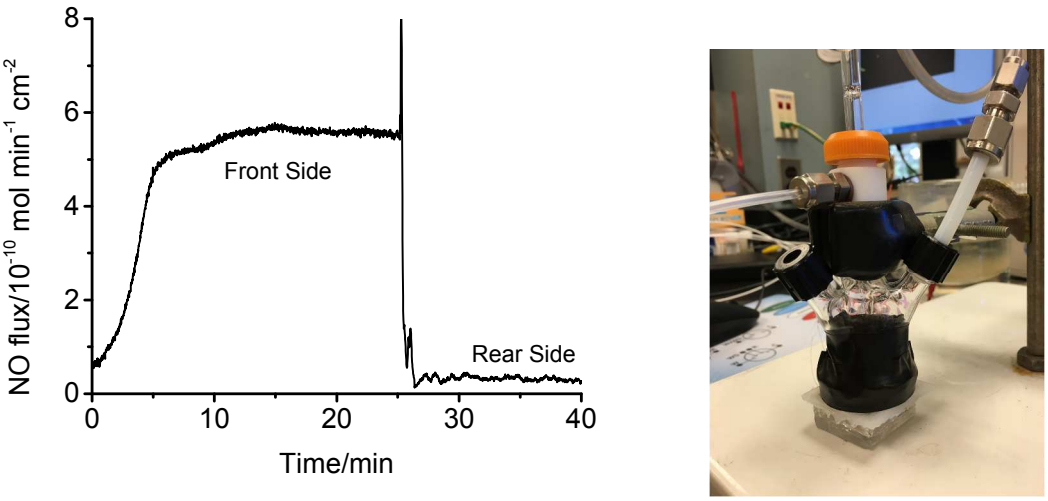

Figure S2. Measurement of asymmetry of NO release from front side and rear side of the wound healing patches. The applied voltage is -0.44 V.