Supporting Information for

In Vivo Analytical Performance of Nitric Oxide-Releasing Glucose Biosensors

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Content

Fabrication and Benchtop Evaluation of NO-Releasing Glucose Sensors

Bare sensors were first cleaned by sonication in EtOH. Electropolymerization of phenol onto the working electrode was carried out via chronocoulometry (+900 mV *vs*. Ag/AgCl, 15 min) in a stirred solution of deoxygenated PBS buffer containing 40 mM phenol. The total charge passed was measured to be $-1.64 \pm 0.18 \times 10^{-3}$ C cm^{-2}. Following electrodeposition of the inner-most polyphenol layer, sensors were sterilized in CIDEX PLUS® 28 Day Solution per the manufacturer's instructions and rinsed with sterile water. All subsequent fabrication steps were carried out in a sterile laminar flow hood. Glucose oxidase was immobilized on the sensing surface by encapsulating the GOx in a methyltrimethoxysilane (MTMOS) xerogel membrane. A GOx-containing sol was prepared by mixing 50 μL 120 mg mL^{-1} GOx in H$_2$O with 125 μL 20% v/v MTMOS in EtOH. The addition of water to the alcohol/silane mixture initiates the co-condensation of the silane monomers to form a polymerized silica xerogel that effectively entraps GOx. Sensors were dip-coated 15 times (5-s still time with 10-s drying periods) into the resulting sol and allowed to dry for 30 min. Following deposition of the selectivity and enzyme membranes, sensors were coated with a PU diffusion-limiting/NO-releasing layer similar to steel wire substrates by dip-coating into a particle-containing PU solution. A TPU topcoat was then applied as an additional layer. Control sensors were coated using PU solutions containing MAP3 or MPTMS nanoparticles (72 and 48 mg mL^{-1}, respectively) that were not functionalized with *N*-diazeniumdiolate or *S*-nitrosothiol NO donors. Sensors were stored in individual sterile centrifuge tubes in a vacuum-sealed bag and kept at -20 °C until use.

Biosensor performance was evaluated via chronoamperometry at 37 °C in PBS buffer under conditions of air saturation using a CH Instruments 1030A potentiostat (Austin, TX). Sensors were pre-conditioned by soaking in PBS for 3–4 h prior to testing, and polarized at +600

mV *vs.* Ag/AgCl in PBS for 20 min to hydrate the sensor membranes and achieve a stable background current, respectively. Calibration curves were generated by stepwise increasing the buffer glucose concentration in 3 mM increments (up to 30 mM) under stirred conditions. Sensor response time was determined as the time required to reach 95% of the steady-state current (t_{95}) in response to 5.6 mM glucose under stirred conditions. Amperometric selectivity coefficients for glucose over potential endogenous interfering species (e.g., acetaminophen, ascorbate) were calculated using the equation below, where ΔI_{Glu} and ΔI_j represent the measured current response to step increases in glucose (c_{glu}=5.6 mM) and the interferent (c_j=100 µM) concentration, respectively.

$$\log K_{glu,j}^{amp} = \log\left(\frac{\Delta I_j/c_j}{\Delta I_{glu}/c_{glu}}\right)$$

In Vivo Protocol for Biosensor Implantation and Operation

The animal protocol used in this study was approved by the IACUC at Synchrony, LLC (Durham, NC). The in vivo performance of glucose biosensors was evaluated in Yorkshire-type piglets (n=10; Palmetto Research Swine; Reevesville, SC) weighing approximately 7–15 kg. Pigs were anesthetized using Telazol (2–6 mg kg^{-1} intramuscular) and maintained on isoflurane (2–3% v/v balance O_2) during sensor implantation. The dorsal skin was prepared by clipping of the hair and alternating iodine and alcohol scrubs, each in triplicate. Six sensors (three NO-releasing, three control) were implanted in pairs (one NO-releasing, one control) spaced ~3 cm by cannulation into the subcutaneous space using a sterile 22-gauge needle. Sensors were positioned approximately 4 cm lateral and perpendicular to the spine and 12–30 cm caudal to the scapulae. Dermabond^{TM} was used to secure the sensor at the entry wound. The sensors were further secured using Prolene 3-0 sutures, gauze, and Opsite®. Sensor pairs were connected to battery-operated wireless bipotentiostats (model 8100 K-5, Pinnacle Technology, Inc.; Lawrence, KS) to allow free motion of the animal while applying a constant +600 mV (*vs.* Ag/AgCl) to the working electrode. The bipotentiostats transmitted current measurements wirelessly via an integrated RF transmitter to a nearby receiver. Data was collected in real time using Sirenia acquisition software (Pinnacle Technology, Inc.; Lawrence, KS).
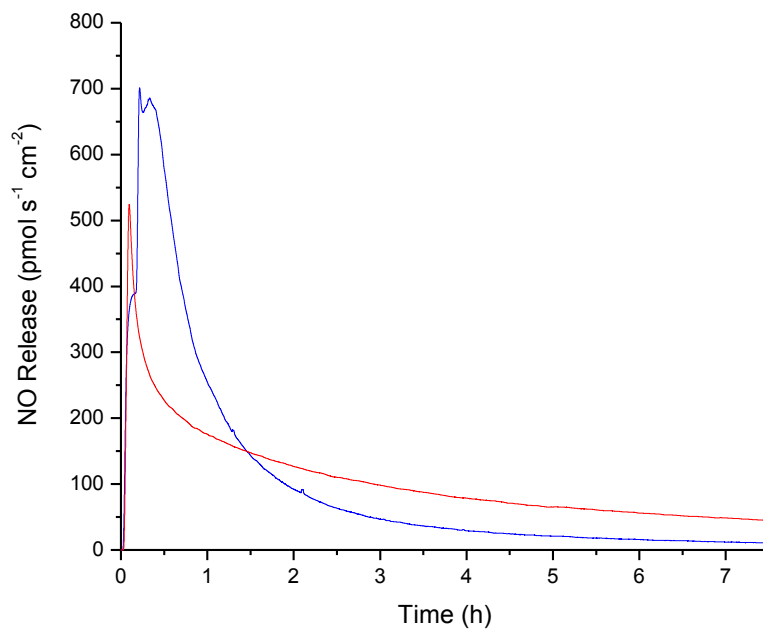
Figure S1. Nitric oxide release from MPTMS-RSNO (red) and MAP3/NO (blue) coatings. For MPTMS-RSNO coatings, NO measurements were performed in a light-shielded flask and in the presence of DTPA.
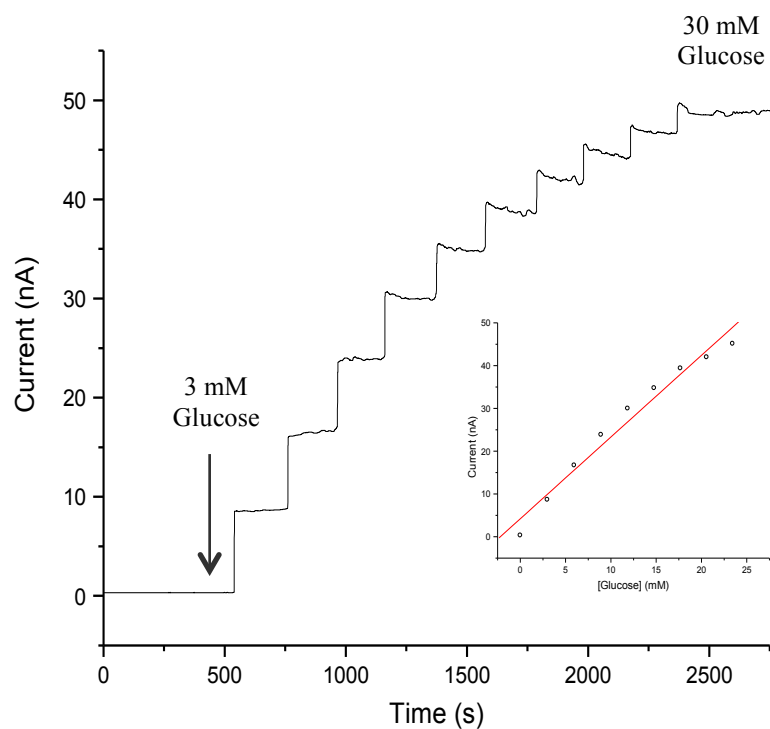
Figure S2. Amperometric response for NO-releasing PU-coated needle type glucose sensor after pre-conditioning in PBS. Inset represents a typical calibration curve after PU membrane hydration.
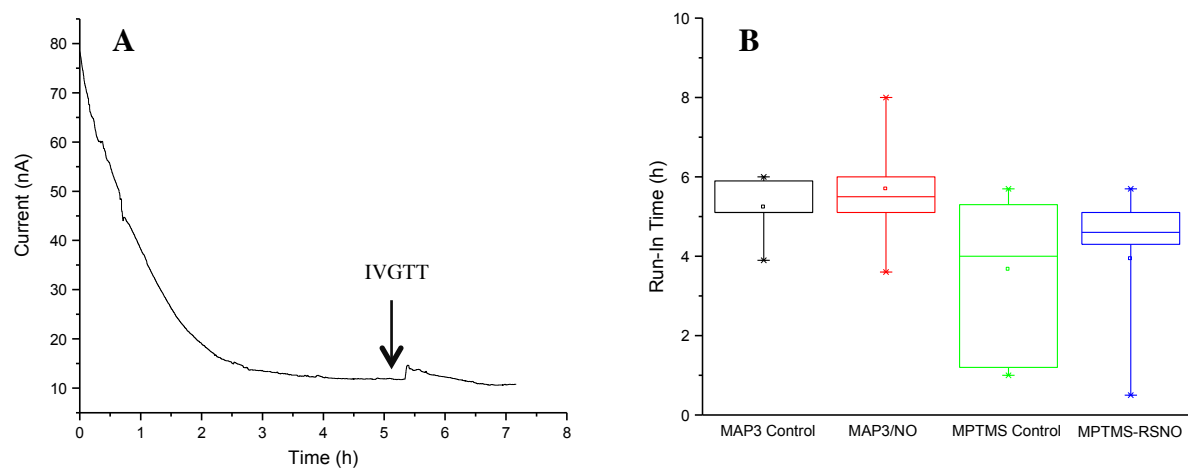
Figure S3. (A) Representative current trace for glucose biosensor following implantation and (B) distribution of estimated run-in times for NO-releasing and control sensors. Error bars indicate the total spread of data and boxes represent data points that lie in the center quartiles (25–75%).
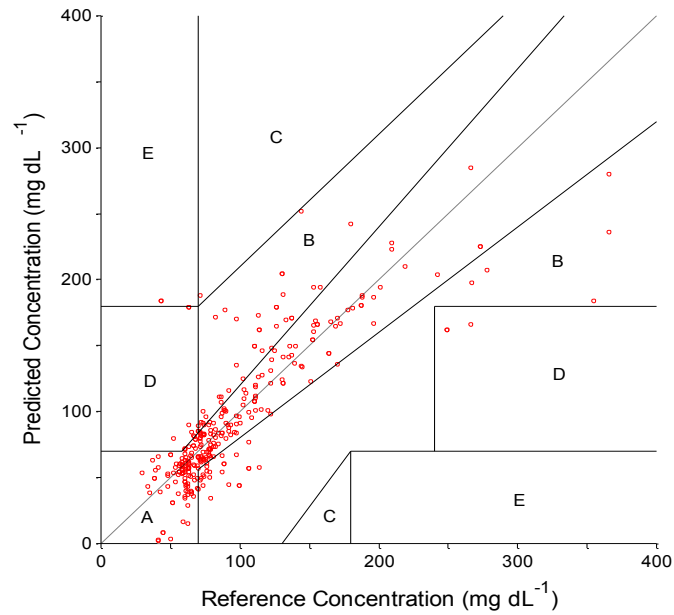
Figure S4. Clarke error grid for MPTMS-RSNO biosensors on day 0. While daily IVGTT provided excursions into the hyperglycemic range, the majority of glucose determinations (~70%) were made in the 50–100 mg dL^{-1} range. Zones labeled A and B represent clinically acceptable blood glucose measurements, while zones C, D, and E represent erroneous and progressively worse determinations.
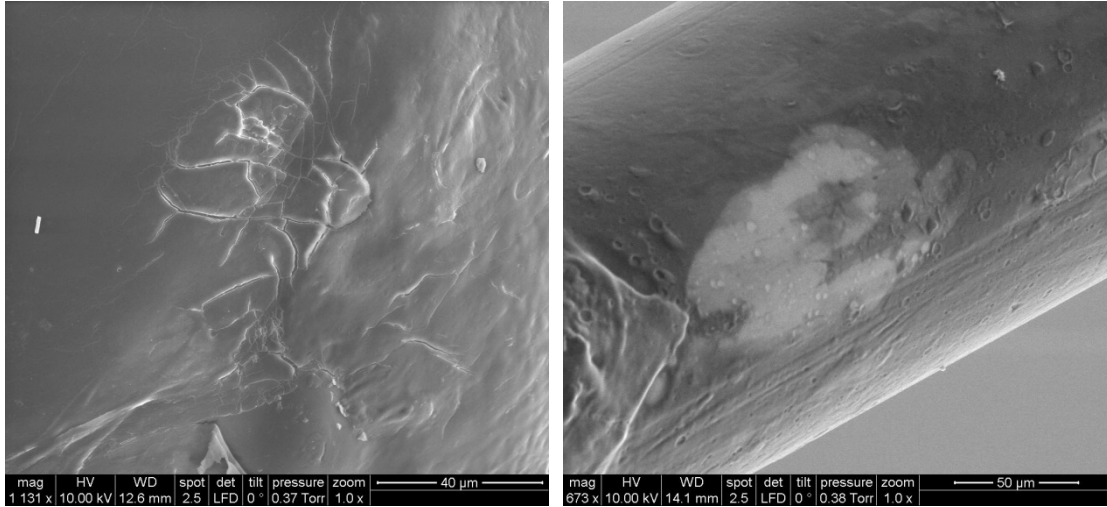
Figure S5. Representative post-explantation scanning electron micrographs of glucose biosensor working electrode surfaces exhibiting (A) membrane cracking and (B) partial coating delamination.

Table S1. Summary of in vitro sensor analytical merits

Performance Merit	Day[a]	MAP3 Control	MAP3/NO	MPTMS Control	MPTMS-RSNO
	0	2.3±0.2	2.3±0.1	2.1±0.4	2.2±0.7
	1	2.2±1.0	2.4±1.8	2.7±0.1	2.1±0.9
Sensitivity (nA mM^{-1})	3	1.3±0.3	1.5±1.3	2.2±1.0	2.0±1.4
	7	1.6±0.1	1.8±1.1	2.0±0.5	1.8±0.3
	10	1.4±1.0	1.9±1.5	1.3±0.3	1.4±0.8
Response Time (s)	0	28±18	13±9	36±12	40±27

[a]Sensitivity of NO-releasing and control glucose biosensors was evaluated after incubation in PBS at 37 ºC

Table S2. Nanoparticle NO Donor Characterization

Nanoparticle NO Donor	$[NO]_T$ ($\mu mol\ mg^{-1}$)	Particle Diameter (nm)[a]
MAP3	2.03±0.20	820±70
MPTMS	3.36±0.62	620±80

[a]Nanoparticle diameter estimated via scanning electron microscopy