Supplementary Information

A Differential Dielectric Affinity Glucose Sensor

Xian Huang[1], Charles Leduc[2], Yann Ravussin[2], Siqi Li[3], Erin Davis[3], Bing Song[1], Dachao Li[4], Kexin Xu[4], Domenico Accili[5], Qian Wang[3], Rudolph Leibel[2], and Qiao Lin[1*]
[1]Department of Mechanical Engineering, Columbia University, New York, NY 10027, USA, [2]Department of Pediatrics, *Columbia University Medical Center,* New York, NY 10032, USA, [3]Department of Chemistry and Biochemistry, University of South Carolina, Columbia, SC 29208, USA, [4]College of Precision Instrument and Opto-electronics Engineering, Tianjin University, Tianjin, 300072, China, [5]Department *of Medicine, Columbia University Medical Center,* New York, NY 10032, USA.
*Corresponding author: Prof. Qiao Lin, email:qlin@columbia.edu, 236 S.W. Mudd, New York, NY 10027 USA, Phone: (212) 854-1906

Fabrication Processes of the Differential Dielectric Sensor

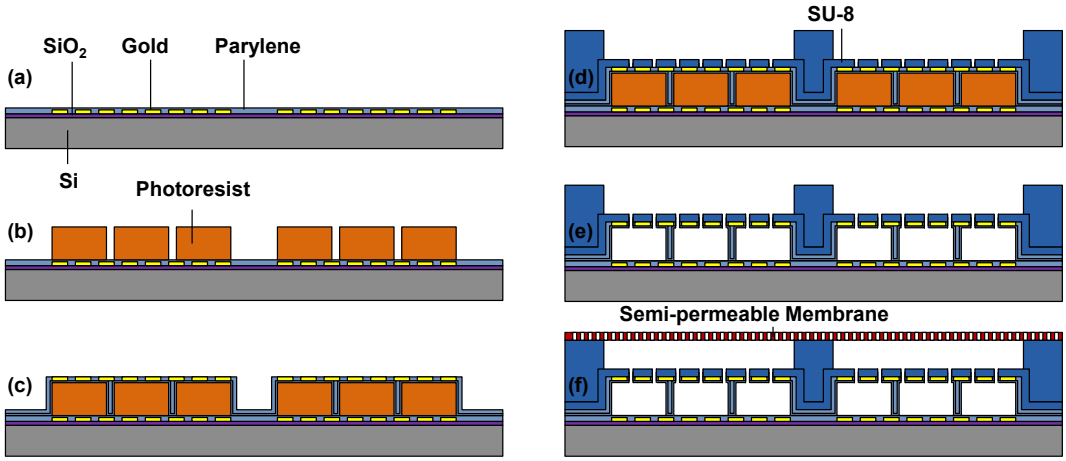

Fig. S1. Fabrication processes: (a) Gold layer deposition and patterning to form bottom gold electrodes, and passivation of the electrodes by parylene; (b) Sacrificial photoresist layer deposition and patterning; (c) Parylene deposition and gold layer deposition and patterning to form perforated electrodes; (d) SU-8 deposition and patterning to form diaphragms and microchambers; (e) removal of sacrificial photoresist layer; (f) bonding of a semi-permeable membrane to seal the microchambers.

The processes and materials used to fabricate the sensor were similar to those described elsewhere[1]. Briefly, chrome (Cr)/gold (Au) (5/100 nm) were deposited and patterned to form the lower electrodes (1 mm × 1mm × 100nm for sensors using in *in-vitro* testing and 0.5 mm × 0.5mm × 100nm for sensors using in *in-vivo* testing) and on-chip resistive temperature sensors (Fig. S1a). Two Parylene layers (1 and 1.5 μm in thickness, respectively) were deposited, sandwiching a sacrificial photoresist layer that was patterned to define the electrode gaps (1 mm × 1 mm × 5 μm each for sensors using in *in-vitro* testing and 0.5 mm × 0.5mm × 5 μm for sensors using in *in-vivo* testing) (Fig. S1b). Cr/Au (5/100 nm) were then deposited and patterned to form the upper electrodes, which were sandwiched between the Parylene layer below and another Parylene layer (3 μm in thickness) deposited above (Fig. S1c). Two SU-8 layers (20 and 80 μm in thickness, respectively) were successively deposited, which along with the Parylene layers sandwiching the upper electrode, were patterned to form the perforated diaphragms (6 × 6 arrays of through openings with 50 × 50 μm in dimension and 150 μm in spacing)

and the microchambers (Fig. S1d). After removal of the sacrificial layer to release the diaphragms (Fig. S1e), the microchambers were each capped by a cellulose acetate semi-permeable membrane by adhesive bonding (Fig. S1f).

Experimental Setup

Two experimental setups have been used to characterize the sensor, one allowing dielectric measurement at a range of frequencies, while the other focusing on measurement at a fixed frequency. First, a capacitance/voltage converter circuit[2] measured individually the frequency response of both the sensing and reference polymers. That is, this circuit measures the capacitance of the sensing or the reference module under an AC electrical field (e-field) with an amplitude of 10 mV and a frequency varied from 0.5 to 100 kHz. The amplitude and the phase of the output voltage from the circuit were captured by a lock-in amplifier (SR830, Stanford Research Systems) to calculate the sensor capacitance using previously reported equations[2]. The second setup focused on differential capacitance measurements at a fixed frequency (32 kHz) and was hence more simplified, using a Σ-Δ capacitance digital convertor (CDC) (AD7746, Analog Devices) with a resolution of 4 aF and an accuracy of 4 fF. To measure the differential capacitance, the CDC applied an AC excitation voltage at 32 kHz to the lower electrodes, while the upper electrodes in the diaphragms were connected to differential capacitance measurement pins of the CDC.

Materials

Poly(N-hydroxyethylacrylamide-*ran*-3-acrylamidophenylboronic acid) (PHEAA-*ran*-PAAPBA), whose properties and ability in affinity glucose detection has been previously demonstrated[3], was used as the glucose sensing polymer. Poly(acrylamide) (PAA), which does not react with glucose[4, 5], was used as the reference polymer. D-(+)-glucose were purchased from Sigma-Aldrich. A buffer solution was prepared using diluted Ringer's stock solution (Nasco). The sensing and reference solutions were prepared by dissolving 284 mg PHEAA-ran-PAAPBA and 142 mg PAA, respectively, in the buffer solution (10 mL) with proper agitation. A series of glucose solutions with varying concentrations (50, 100, 200, 300, 400, and 500 mg/dL) were prepared by dissolving appropriate amount of glucose in 100 mL buffer solution.

Steady-State Frequency Response at Different Glucose Concentrations

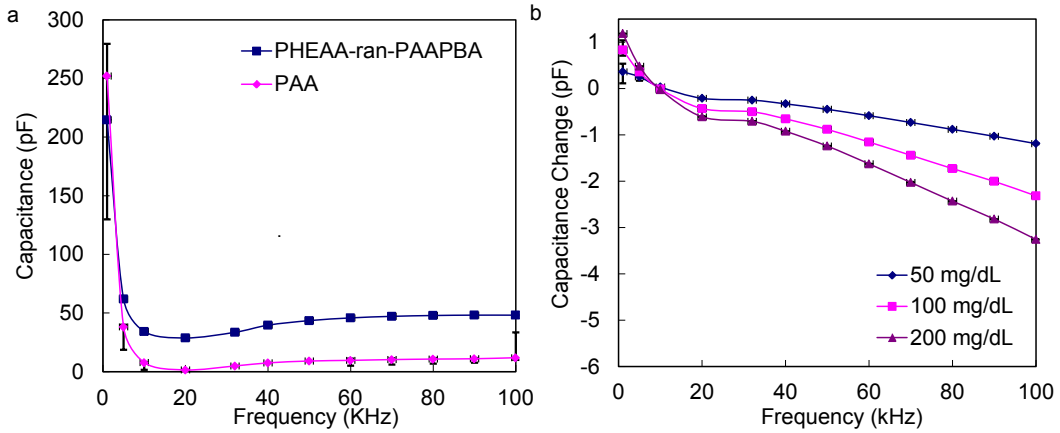

Fig. S2. (a) The frequency responses of the PHEAA-*ran*-PAAPBA polymer solution and PAA polymer solution at 0 mg/dL glucose concentration; (b) Capacitance change of the sensor filled with PHEAA-*ran*-PAAPBA solution at varying glucose concentration with respect to the capacitance at 0 mg/dL glucose concentration.

In vivo experiments

Preliminary *in vivo* testing of the device was conducted at the Columbia University Medical Center in 10-week old C57BL/6J laboratory mice using an approved protocol. Under anesthesia by inhaled isoflurane (3-3.5%) in oxygen, the skin of the mice was shaved, and then washed with betadine and alcohol, followed by incision of 1.5-2 cm caudal to the interscapular region. Small pouches were made through a hemostat at the location where the sensors were implanted. Blood glucose concentrations were manipulated by intraperitoneal (IP) administration of glucose (2 g/kg) and insulin (0.25 unit/kg). Blood glucose concentrations were first allowed to equilibrate for one hour with no exogenous injections of glucose or insulin, followed by hypoglycemia induced by IP insulin administration and hyperglycemia via glucose administration. The implanted glucose sensors continuously measured the glucose concentrations in interstitial fluid (ISF), while the reference capillary blood glucose concentrations were monitored with a commercial glucose meter (Freestyle Lite, Abbott Diabetes Care) by tail nicking at specified frequencies.

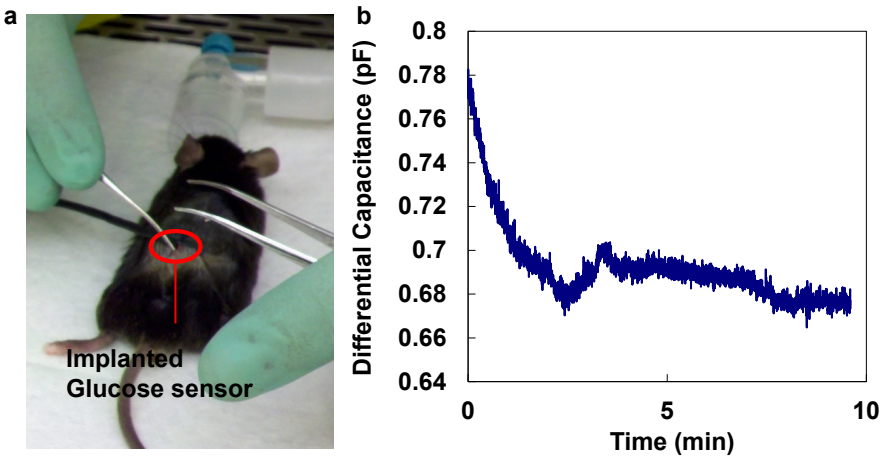

Fig. S3: Preliminary animal experiment. (a) Sensor implantation in a laboratory mouse; (b) A typical differential capacitance change of the implanted sensor during the initialization.

Reference:

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