Supporting Information

Transcutaneous Flexible Sensor for In Vivo Photonic Detection of pH and Lactate

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Experimental Procedure:

**Oxygen-Sensitive Dye-Coated PTFE Sheet Fabrication:**
Oxygen-sensitive dye solution was created by mixing 4 mg of PtTPTBP dye (Frontier, USA), 60 mg of polystyrene (molecular weight: 2500, Sigma-Aldrich, USA) and 900 µL of chloroform (Sigma-Aldrich, USA) in a 1.5 mL amber vial. The resulting concentrations are 440.873 µM PtTPTBP and 0.024 mM polystyrene in 900 µL chloroform. 200 µL of this solution was pipetted onto a circular PTFE sheet. The chloroform was allowed to evaporate resulting in the oxygen-sensitive dye-coated PTFE sheet. This dye-coated PTFE sheet served as the base of both the LOX and Oxygen Sheets.

**LOX Sheet Solution Preparation:**
The LOX sheet is formulated using two solutions: (1) Protein Mixture and (2) Pretreatment Solution. Protein Mixture is composed of 0.060 mM LOX (LCO-301, 108U/mg, 0.0096 mg/µL, Toyobo, Japan), 193.13 mM poly(ethylene glycol) dimethacrylate 2000 (PEGDMA2000, 0.386 mg/µL, Sigma-Aldrich, USA), 43.33 mM Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP, 0.0127 mg/µL, Sigma-Aldrich, USA), 1.16 mM catalase (AN366A, 5410U/mg, 0.28 mg/µL, BBI Solutions, U.K.), and 1X Phosphate Buffered Saline Solution (1X PBS). Pretreatment Solution is composed of 75% v/v poly(ethylene glycol) diacrylate 400 (PEGDA400, Polysciences, USA), 25 % v/v Milli-Q water, and 28 mM LAP (0.00825 mg/µL).

**LED Spectra Acquisition System**
To acquire LED spectra, a caged system containing a suspended optical fiber was created. All items were procured from ThorLabs (USA). A Blank Cage Plate (LCP03) was used as the base of the system. Assembly rods SR05, SR1, and SR1.5 rods were used to build vertically. Vertical mount plate CPVM threaded with a SM1SMA fiber adapter was used to incorporate jacketed fiber patch cable M92L01 into the system. The distance between the M92L01 aperture and the blank cage plate was approximately 90 mm. The opposite end of the jacketed optical fiber was connected to a CCS200 spectrometer. MSF with LEDs were taped down and placed in the center of the blank cage plate such that upon LED activation, M92L01 would capture emitted light. ThorLabs Optical Spectrum Analyzer (OSA) software was used to acquire spectra. Unless otherwise stated, integration times were set to 100 ms. To acquire data, LabView code (National Instruments, USA) was used to turn on the MSF LEDs and spectra were then obtained using the LED Spectra Acquisition System. Drive currents for each LED were less than 10 mA.

**Sensor Film Application**
The HSS was the first sensor sheet applied. After swelling, the HSS was removed from Milli-Q water and placed onto a glass slide. A razor blade was used to remove hydrogel from the sheet edges to expose underlying PTFE. This process resulted in a rectangular hydrogel with dimensions of roughly 1 cm x 0.8 cm. Loctite 4981 was then applied along the width of the MSF, between the 465 nm LED and the top most 625 nm LED. The shorter side of the HSS (0.8 cm) with exposed PTFE was laid down onto the Loctite 4981, with the resin-tethered HPTS side facing the LEDs. The Loctite 4981 (Henkel, Germany) was then allowed to cure. The MSF was then flipped over, and Loctite 4981 was applied to the top edge and along the lateral edges of the back of the MSF. The remaining exposed PTFE edges of the HSS were then pressed in contact with the Loctite 4981 and allowed to cure. After HSS was cured onto the MSF, LOX and Oxygen Sheets were applied onto the MSF. The sheets were cut into smaller sheets (1.8 mm x 1.5 mm) immediately following polymerization and adhered directly onto the 625 nm LEDs with Loctite 4981. When all sensing sheets were applied, the MSF was allowed to incubate in 1X PBS for at least 8 h at room temperature prior to testing.

**Benchtop Optical System Set-up**
The benchtop optical system was built using ThorLabs (USA) assembly rods (SR05, SR1, and SR1.5 rods) with vertical cage system mounting plate CPVM (ThorLabs, USA) serving as the base component. Building from bottom to top, cage plate LCP01T (ThorLabs, USA) was suspended above the CPVM to add on a jacketed multi-mode optical fiber patch cable (0.22 NA, 200 µm core, M92L02, ThorLabs, USA) coupled to a collimator (F810APC-543, ThorLabs, USA). The opposite end of the optical fiber patch cable was connected to a CCS200 spectrometer (ThorLabs, USA). Above the collimator, a LCP02 (ThorLabs, USA) cage plate adapter housing a 5x objective lens (0.10 NA, Newport, USA) was added to capture HSS-emitted light. Settled above the objective lens on an additional LCP02 was a Perma-Proto board (Adafruit, USA) with 400 and 465 nm dominant-wavelength LEDs soldered on opposite ends of a drilled hole (diameter = ¼ inch). In parallel, exposed PTFE of an HSS was used to adhere the sheet onto a µ-Slide 2 well (Ibidi, USA) using Loctite 4981. The adhesive was allowed to dry for at least 5 min prior to testing. The well was secured above the protoboard using tape. The well was positioned,
such that upon testing, the HSS sat over the LEDs and emitted light was collected through the drilled hole with the 5x objective lens. ThorLabs OSA software was used to acquire spectra data. An Arduino Uno (Arduino, USA) with custom Arduino software (Arduino, USA) was used to power the LEDs (5V output). Resistors (±5% tolerance range, BOJACK, China) with total resistance values of 1450 and 830 ohms were placed in series with the 400 and 465 nm LEDs, respectively. Integration time for both LEDs was 1s. For pH testing, the HSS was first washed with the first pH test solution 10 times and then allowed to equilibrate for 30 s. 3 emission spectra were recorded for each LED and averaged after background spectrum (no LED excitation) subtraction. The background-subtracted emission spectrum was then saved as a text file. To exchange pH solution, the solution previously tested was aspirated under vacuum and the next pH test solution was tested as previously mentioned. MATLAB (MathWorks, USA) was used for subsequent analysis.

**pH Solution Preparation, Validation, and Testing**

pH test solutions were prepared following Sigma-Aldrich’s Phosphate Buffer Preparation Table, using potassium phosphate monobasic anhydrous (795488-500G, Sigma-Aldrich, USA), sodium phosphate dibasic heptahydrate (S9390-1KG, Sigma-Aldrich, USA), and Milli-Q water. Polynomial equations were fitted onto both reagent quantities listed in the Preparation Table to interpolate pH solution formulations. The pH solutions were probed using a Mettler Toledo FiveEasy pH probe (Sigma Aldrich, USA). pH probe calibrations were completed with pH 4.01, 7.00, and 10.01 buffer solutions (Orion™ Standard All-in-One™ pH Buffer Kit, 910199, ThermoFisher Scientific). Only calibrations with slopes greater than 95 were used. For pH testing *in vitro*, measurements were obtained at room temperature. For PALS pH testing *in vitro*, each MSF was placed in a 20 mL scintillation vial and incubated in the series of pH solutions. For testing, except for rise times studies, the MSF was first washed with the pH test solution 10 times and then allowed to equilibrate for 30 s. After measurements were obtained, the test solution was aspirated. This process was completed for each new test solution. For rise time studies, only 1 wash was completed when introducing the next test solution.

**Lactate Solution Preparation, Validation, and Testing**

Lactate test solutions were prepared with L- (+)-Lactic acid solution (27714-1L, Sigma Aldrich, USA) and 1X PBS. pH adjustments were made with 0.1 M HCl and 0.1 M NaOH (43617, Sigma-Aldrich). 0.1 M HCl was formulated by diluting 11 N HCl (A144C-212, Fisher Scientific, USA) with Milli-Q water. Unless otherwise noted, pH of lactate solutions was adjusted to 7.45. The lactate solutions were verified using a YSI 2300 STAT Plus Glucose and Lactate Analyzer (Yellow Springs Instrument, USA). For lactate testing *in vitro*, measurements were obtained at room temperature. Each MSF was placed in a 60 mm plastic dish (BD Falcon, USA) and incubated in the series of lactate solutions. For testing, except for rise times studies, the MSF was first washed with the lactate test solution 10 times and then allowed to equilibrate for 30 s. After measurements were obtained, the test solution was aspirated. This process was completed for each new test solution. For rise time studies, only 1 wash was completed when introducing the next test solution.
Figure S1. Micrograph of a Filter-Coated Photodetector. Scale bar = 1 mm. Primary Green filter applied onto a SFH2716 photodiode (OSRAM Opto Semiconductors, Germany).

Figure S2. Simplified Schematics of PALS Printed Circuit Boards. A) High side control of the charlieplex circuit is achieved with a multiplexer (SN74CBTLV3251PWR, Texas Instruments, USA), which toggles the output line of a square wave generated by the Teensy 3.2. Low side control is achieved by a constant sink LED driver (TLC5940, Texas Instruments, USA). The sink was alternated over 3 lines to control 4 LEDs on the MSF. B) Integrator board utilizing 100pF internal capacitance. Timing of IVC102 integration is controlled by the Teensy 3.2 via digital outputs. IVC102 output signal is run through a voltage divider (not shown) before being read at the analog input of the Teensy 3.2. C) Photodetector board
utilizing a S6775-01 photodiode (Hamamatsu, Japan). Output signals from the op amp are read using an analog input of the Teensy 3.2. D) LED charlieplexing schematic. E) Fully connected PALS.

Figure S3. Normalized LED Emission Spectra. Signals were obtained using the LED Spectra Acquisition System. Spectra were corrected for any background signals. The 400, 465, and 625 nm LEDs have peak emission wavelengths of 400, 467, and 631 nm, respectively.

Figure S4. Baseline pH Sensor Stability in pH 7.45. PALS pH sensor measurements over 8 h.

Figure S5. Spectral Stability of the pH Optode LEDs. Spectra were obtained every 3 min for 30 min with the LED Spectra Acquisition System. Each spectrum is an average of 3 measurements after background signal subtraction. A) Overlapped 400 nm LED spectra. Inset: AUC analysis of wavelengths encompassing the peak emission wavelength show minimal
variance. B) Overlapped 465 nm LED spectra. Inset: AUC analysis shows minimal variance. C, Top) 465 nm LED peak emission intensity at 467 nm shows no significant change over time. C, Bottom) 400 nm LED peak intensity is stable.

Figure S6. Baseline Lactate Sensor Stability. Lactate sensing in 4 mM lactate over 4 h.

Figure S7. Retrospective Linear Regression Models. Standard error of the estimates are (A) 0.017 and (B) 0.20 for the pH and lactate sensor, respectively

Supporting Video S1 – This video shows serial MSF LED activation in vitro.

Supporting Video S2 – This video shows serial MSF LED activation in vivo.