Wearable soft electrochemical microfluidic device integrated with iontophoresis for sweat biosensing

Gulcin Bolat[a], Ernesto De la Paz[a], Nathalia F. Azeredo,[a,b] Michael Kartolo[a], Jayoung Kim[a], Andre Neirdert de Loyola e Silva[a], Ricardo Rueda[a], Christopher Brown[a], Lúcio Angnes,[b] Joseph Wang,[a*] and Juliane R. Sempionatto[a]*

a. Department of NanoEngineering, University of California, San Diego, La Jolla, CA, 92093, United States.
b. Department of Fundamental Chemistry, Institute of Chemistry, University of Sao Paulo, Sao Paulo, Brazil
* Corresponding author: josephwang@ucsd.edu and jsemp@caltech.edu

Supporting Information

Experimental Details

Fabrication of Flexible PDMS Microfluidic Channels and Multilayers

The microfluidic silicon mold was fabricated according to previous work [22]. Shortly, a 50 nm layer of Cr was deposited (Temescal BJD 1800) on a 4" silicon wafer to act as an etch mask. Next, photolithography was used to pattern the microfluidic channels and the unmasked portions were etched by Deep Reactive Ion Etching (Plasmalab Oxford P100), yielding 100 μm tall patterns. Depth measurements were performed using a Dektak 150 surface profiler (Veeco, Plainview, NY). Then, a 70 nm layer of poly(methyl methacrylate) (PMMA 950 A2, MicroChem, USA) was spin-casted onto the Si master, followed by soft baking at 180 °C. A silicone layer of 500 μm (Dow Corning, Sylgard 184) was then spin-casted onto the Si master to yield the final microfluidic

pattern **(Figure S1**). Subsequently, for the pillar inlets, a flat PDMS (10:1) layer, of 1 mm height, was fabricated on a PMMA treated glass slide. A circular puncher of 5 mm diameter was used to produce a cylindrical pillar with 5 mm diameter and 1 mm heigh. The pillars were aligned and attached to the inlets of the microfluidic features, by casting a thin PDMS layer and curing at 120^{o}C (**Figure S2**). After attachment, the pillar and fluidic layers were punched together using a 700 μm mechanical puncher, to create the openings for sweat collection. Later, the resultant PDMS structure was exposed to UVO ozone (Jetline Co., Irvine, CA) at a gas flow rate of 3 sccm for 10 min (25^{o}C) to be bounded to a flat PDMS layer containing the electrode.

Fabrication and Chemical Modification of the Soft Electrochemical Biosensors

The soft electrodes were fabricated according to a previous publication [22]. Briefly, a 70 nm layer of PMMA was spin-casted onto a 4″ Si wafer to serve as a sacrificial layer. Subsequently, polyimide (PI-2545, HD Microsystems, USA) was spin casted to yield a 1.6μm film on the PMMA layer, followed by soft baking at 110 and 150 °C on a contact hot plate to remove volatile solvents, and then cured at 250 °C in a vacuum oven for 4h. Layers of Cr (550 nm) and Au (200 nm) were deposited by sputter coating (Denton Vacuum LLC, Discovery 635) and photolithography was used to pattern the sensors.

A second layer of the same polyimide thickness was placed on the sensor's interconnect and Reactive Ion Etching (Plasmalab Oxford P80) was used to etch the polyimide and define the layout of the array, exposing only the bonding pads and the sensors. The PMMA layer was then undercut with boiling acetone to enable the removal of the pattern from the Si wafer using a water-soluble PVA tape (3 M Company, Maplewood, MN). The exposed back surface of the electrode was then mounted on glass slides for deposition of Ti (6 nm)/SiO_2 (60 nm) by sputter coating (Denton Vacuum LLC, Discovery 635). A PDMS layer of 500 μm (Dow Corning, Sylgard 184) was spin casted onto a PMMA treated glass slide. The devices were then transferred onto the PDMS through formation of covalent bonds by condensation reactions between ozone (UVO) treated silicone and SiO_2 on the back side of the devices (**Figure S3**). The tape was dissolved with DI water. The devices were then ready for screen printing.

[22] Martín A, Kim J, Kurniawan JF, Sempionatto JR, Moreto JR, Tang G, et al. Epidermal Microfluidic Electrochemical Detection System: Enhanced Sweat Sampling and Metabolite

Supporting Figures

Fabrication of Flexible PDMS Microfluidic Channels and Multilayers

A 50 nm layer of Cr was deposited on a 4" silicon wafer to act as an etch mask. Next, photolithography was used to pattern the microfluidic channels and the unmasked portions were etched by deep reactive ion etching. Then, a layer of poly(methyl methacrylate) was spin-casted onto the Si master, followed by soft baking at 180 °C. Fınally, a silicone layer was then spin-casted onto the Si master to yield the microfluidic pattern **(Figure S1**).

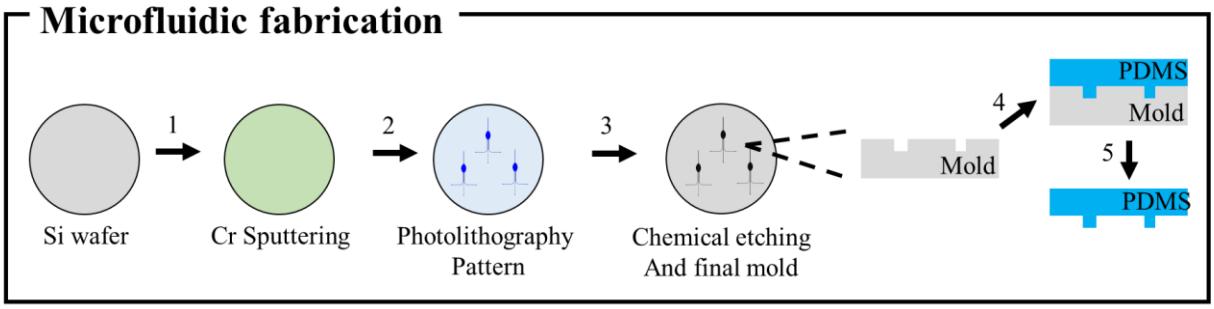


Figure S1. Fabrication of Si master for the PDMS fluidic features.

For the fabrication of the pillar inlets, a flat PDMS layer was fabricated on a glass slide with its surface treated by PMMA. A metallic puncher was used to produce the cylindrical pillars. The pillars were aligned and attached to the inlets of the microfluidic features (**Figure S2**) by using uncured PDMS. After attachment, the pillar and fluidic layers were punched together using a 700 µm mechanical puncher, to create the openings for sweat collection. Later, the resultant PDMS structure was exposed to UVO ozone to be bound to the PDMS layer containing the electrode. (**Figure S2**)

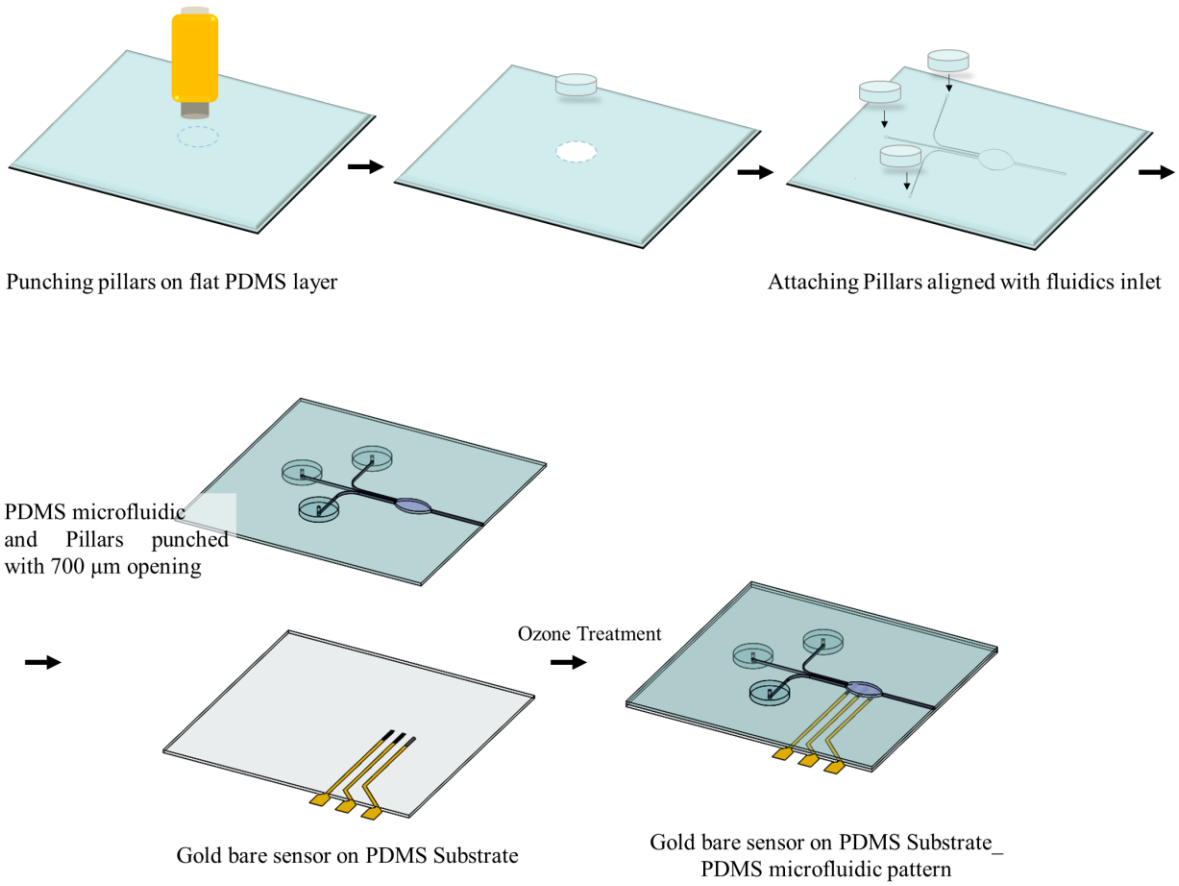


Figure S2. Fabrication of PDMS pillars and assembling to the PDMS fluidic layers

Soft Fabrication of the Electrochemical Biosensor

A layer of PMMA was spin-casted onto a 4″ Si wafer to serve as a sacrificial layer. Subsequently, polyimide was spin casted on the PMMA film. Layers of Cr and Au were deposited by sputter coating, and photolithography was used to pattern the sensors and interconnect. A second layer of polyimide was placed on the sensor/interconnect and reactive ion etching was used to etch the polyimide and define the mesh layout of the array, exposing only the bonding pads and the sensors. The PMMA layer was then undercut with boiling acetone to enable the removal of the mesh from the Si wafer using a water-soluble PVA tape. The exposed back surface of the mesh was then mounted on glass slides for deposition of Ti (6 nm)/SiO_2 by sputter coating. A PDMS layer was spin casted onto a PMMA glass slide. The devices were then transferred onto the PDMS through

formation of covalent bonds by condensation reactions between ozone (UVO) treated silicone and SiO_2 on the back side of the devices. The tape was then dissolved with DI water. The devices were then ready for screen printing **(Figure S3).**

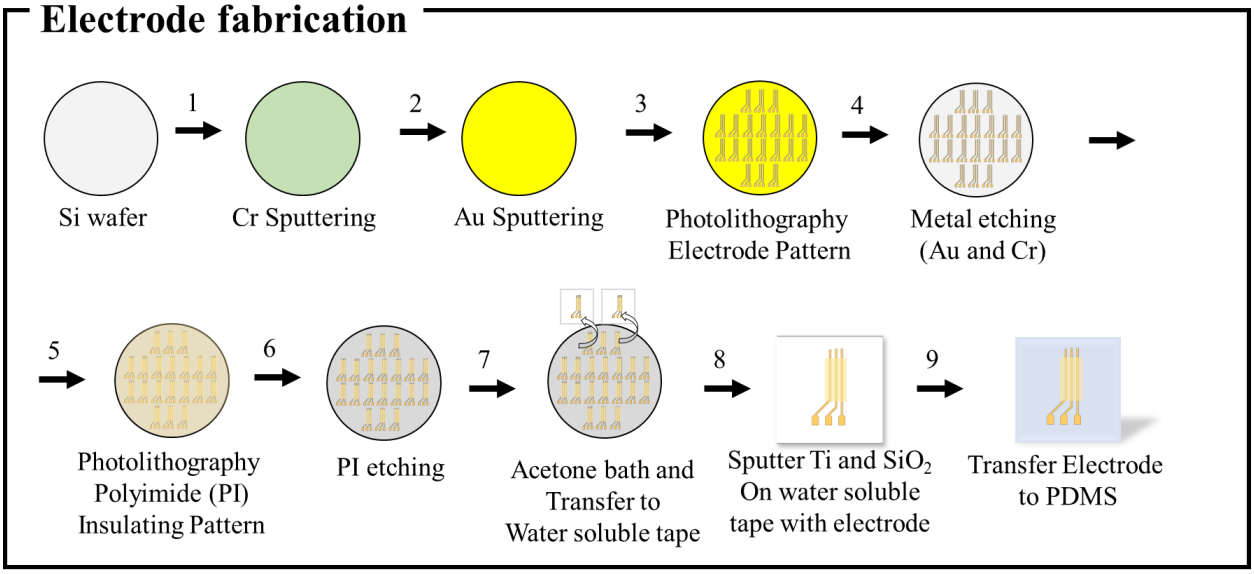


Figure S3. Fabrication steps for soft gold electrodes on PDMS.

The electrochemical sensors were patterned by screen-printing over the microfabricated gold collectors with two separate layers using Ag/AgCl ink as the reference electrode and Prussian blue conductive carbon ink as working and counter electrodes (**Figure S4**). The printed patterns were cured at 80 °C for 10 min in a convection oven.

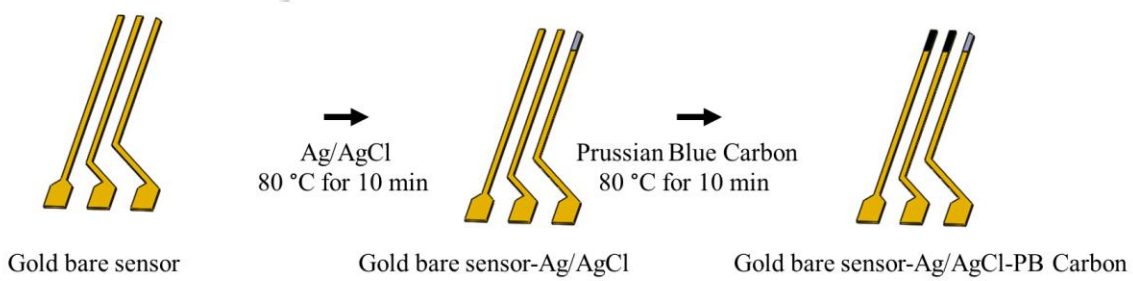


Figure S4. Screen-printing process on the PDMS gold electrodes.

Iontophoretic electrodes fabrication

The iontophoretic electrodes were designed and cut using a Cricut Explore Air Machine. First, a double layer of Ag/AgCl ink was screen printed on a polyimide substrate and cured at 80 ^{0}C for 10 min. After cured, the Ag/AgCl/polyimide- sheet was used as a substrate to cut the electrodes (**Figure S5**).

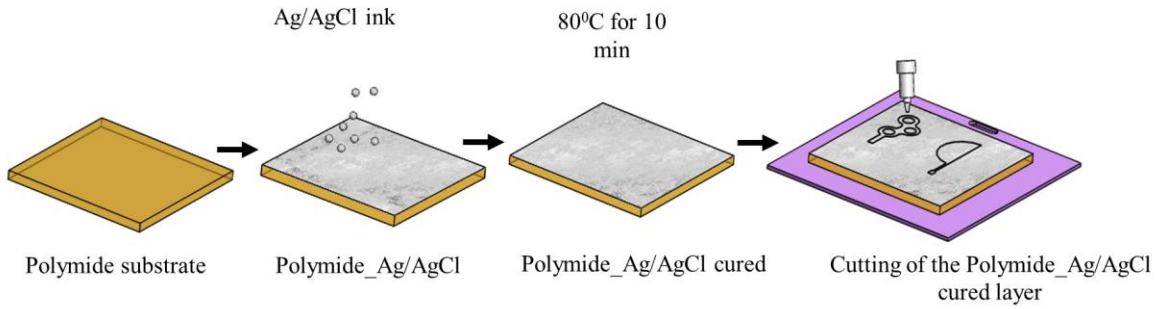


Figure S5. Fabrication of cathode electrodes on polyimide substrate.

Assembling of Microfluidic Device

The iontophoretic microfluidic device was composed of the microfluidics pillar attached to the microfluidic features, which was bonded to the electrode layer (**Figure S6**, top row). Once the PDMS parts were assembled, the anode was placed on the device and an extra PDMS layer was used to improve the adhesion. Adhesive layer was also used on the pillars (**Figure S6** bottom row).

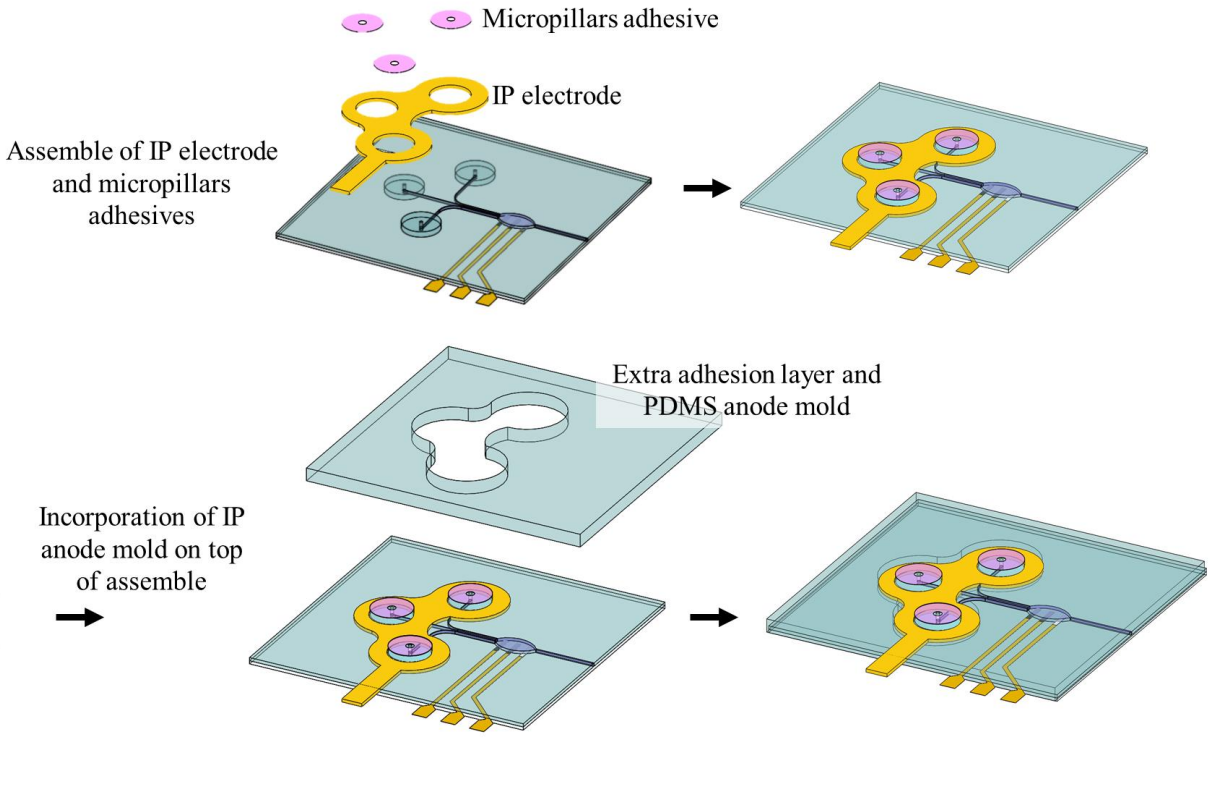


Figure S6. PDMS device assembling steps.

Iontophoretic hydrogels fabrication

The agarose hydrogel was prepared by dissolving the appropriate amount of agarose (4% w/v) in deionized water, heating and continuously stirring at 120 °C. After complete agarose dissolution, pilocarpine nitrate powder was added in the mixture to get 2 % (w/v) pilocarpine in the solution. The solution was then cooled down to 60°C and 120 µL was casted on top of the surface to form a uniform hydrogel layer covering the iontophoretic area (**Figure S7**).

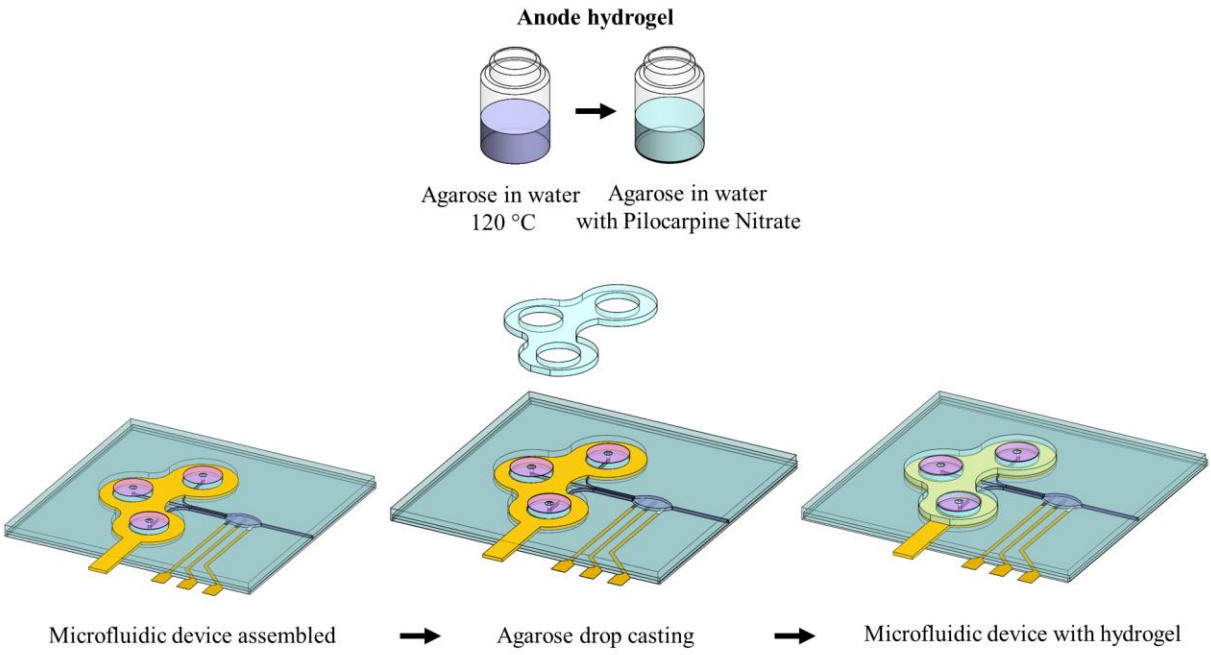


Figure S7. Agarose gel preparation and anode modification.

In the cathodic compartment, a polyvinyl alcohol (PVA) cryogel was used. First, a 5.0 % w/v PVA solution was prepared in deionized water by heating the solution up to 180°C and then cooling it down to room temperature. After the temperature cooling step, the solution was placed in an ice bath (-3°C) followed by pH adjustment to pH 1.0 by adding small aliquots of 5 M Hydrochloric acid solution. Subsequently, an aliquot of 130 µL of glutaraldehyde was added to give a final concentration 50% w/v. The final mixture was then stirred for 1 min and 500 µL were poured into an ecoflex coin shaped mold and set in -20 °C freezer overnight (**Figure S8**). The resulting cryogels were removed from the mold by immersing the mold in deionized water for 30 min.

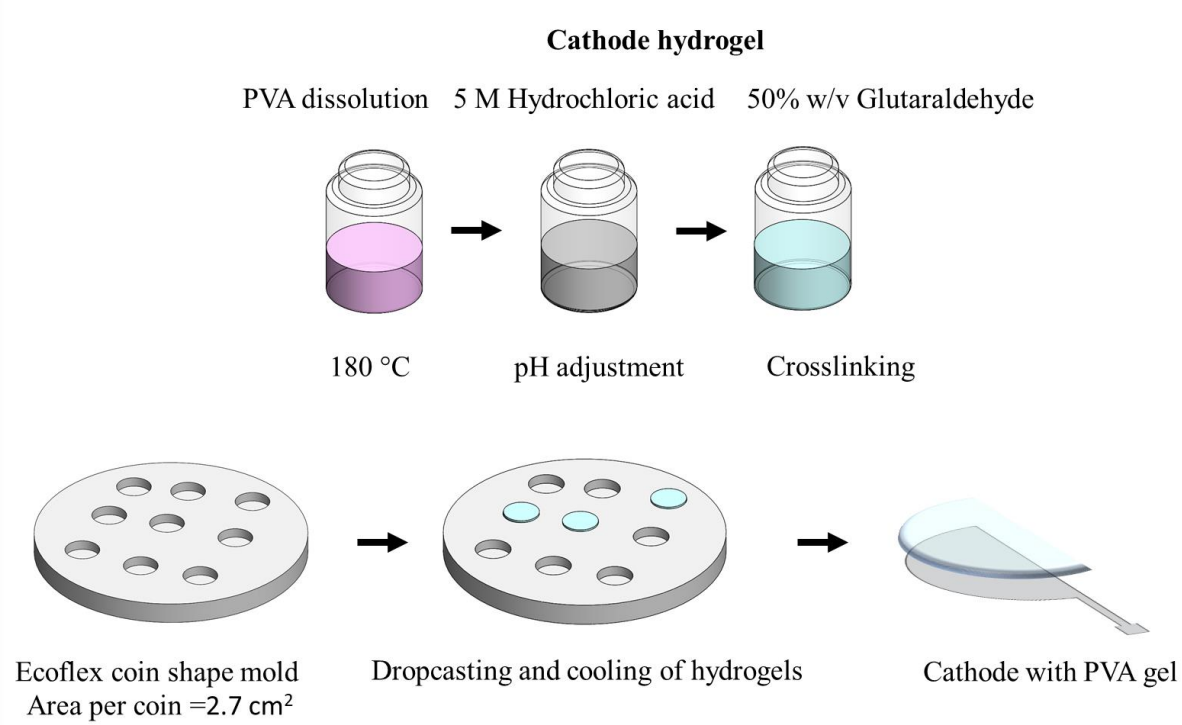


Figure S8. PVA gel preparation and cathode modification.

Device Transfer and On-Body Real-time Glucose Monitoring

The device evaluation on human subjects was conducted in strict compliance following a protocol approved by the Institutional Review Board (IRB) at the University of California, San Diego. The soft IP fluidic device was transferred to the arm of the volunteers, previously cleaned with soap and sterile alcohol prep pads. The microfluidic system was attached to the skin using a Double Coated Medical Tape attached to the PDMS pillars. A PDMS layer, with the same thickness as the pillars, was used to improve the adhesion of the device to the skin (**Figure S9**).

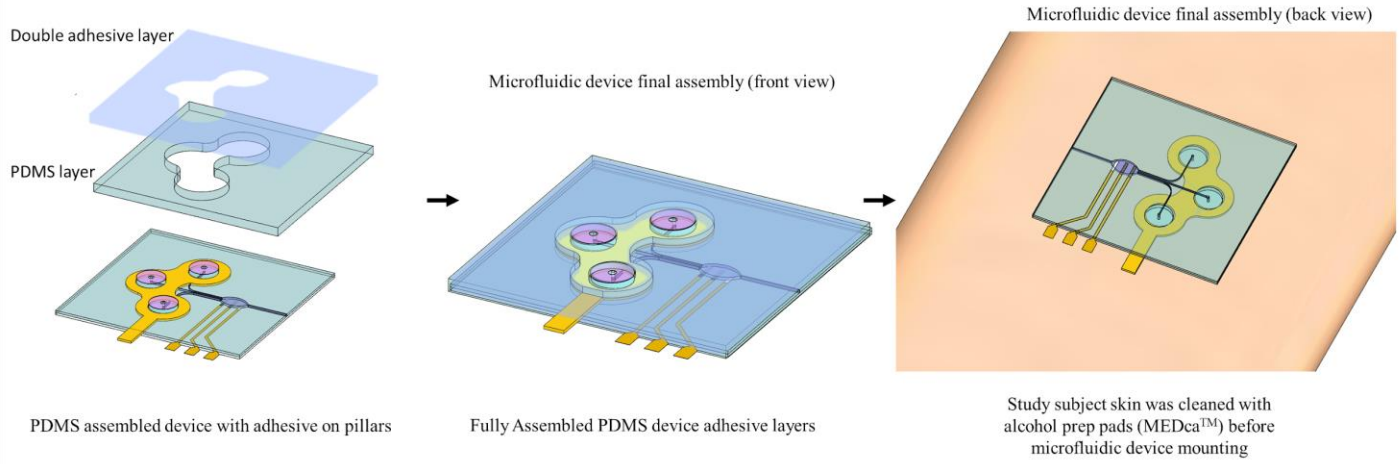
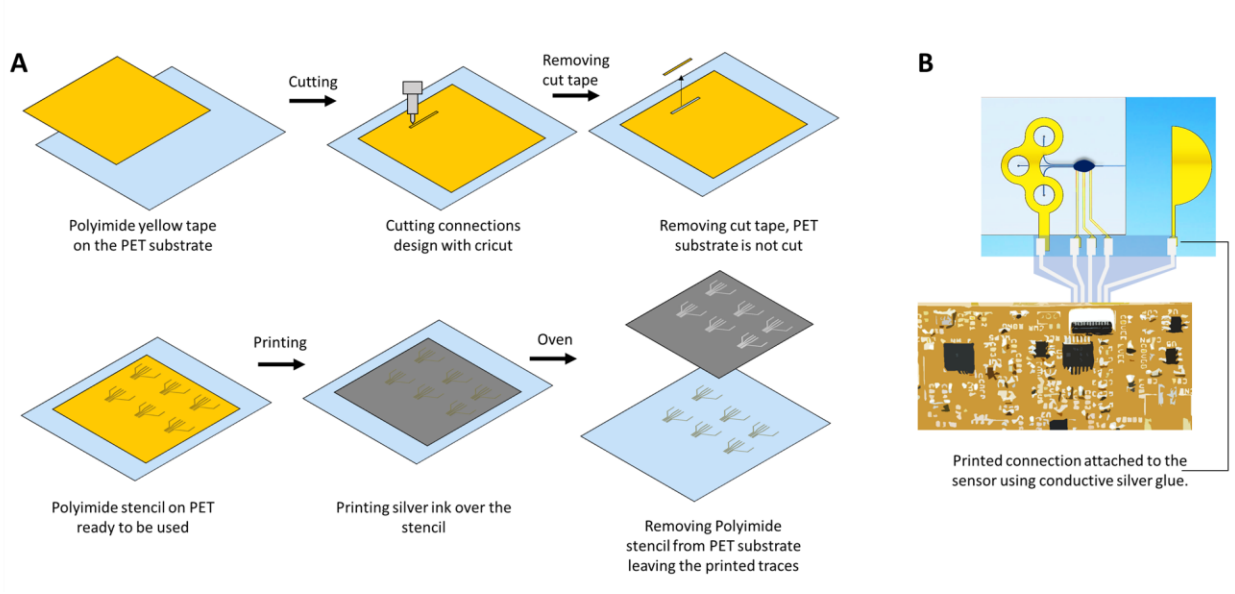


Figure S9. Medical tape assembling and on-body device transfer.

Device Wiring to Electronic Board

The connection of the printed sensors (glucose and IP electrodes) with the electronic board was realized by printing the connector traces. Silver ink was printed on a PET substrate with a custom design to align the connections from the board with the electrodes in the device (Figure S10). The home made stencil was made by cutting a PET coated with a polyimide adhesive tape using a Cricut Explore Air Machine. The printed patterns were cured at 80 °C for 10 min in a convection oven.



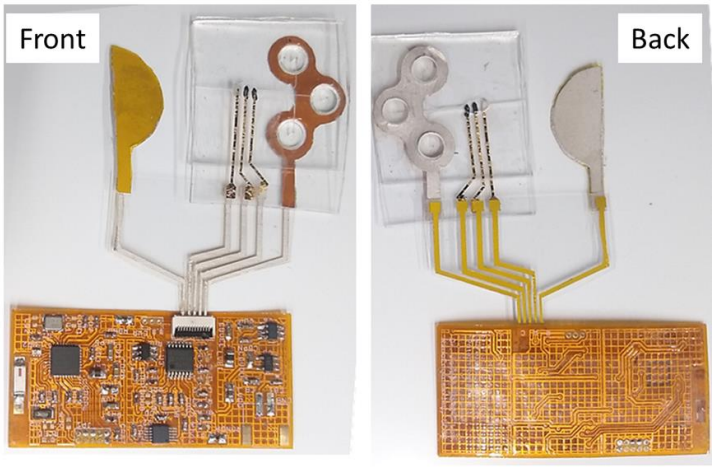


Figure S10. Protocol used to fabricate printed connection for the sensors to the electronic board and photo of the device connected to the electronic board (bottom).

Results and Discussion

The integrated device was able to measure glucose levels in generated sweat successfully, obtaining good correlation values against glucose levels in the blood (**Figure S11).**

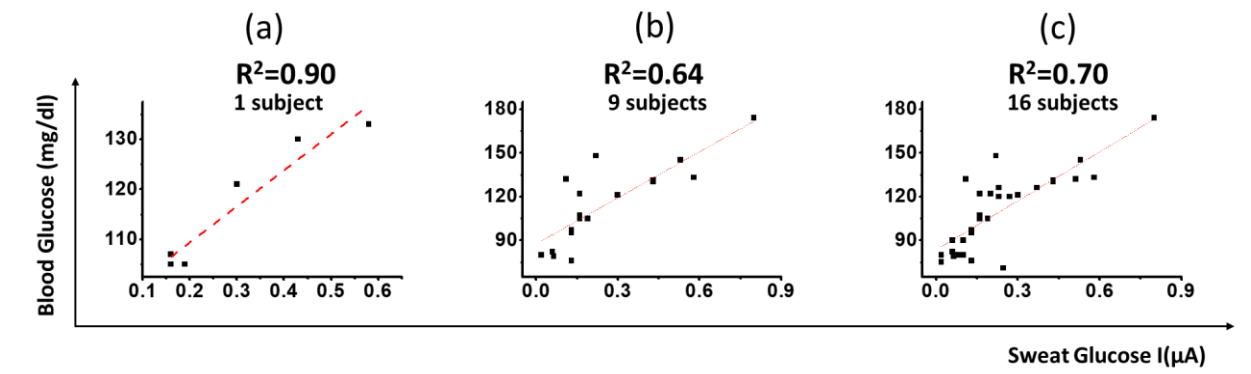


Figure S11. Correlation of blood and sweat glucose for (a) 1 subject with r^2 = 0.9, for (b) nine subjects, with r^2= 0.64 and (c) 16 subjects with r^2=0.7.

Sweat glucose tests without any food consumption showed no significant changes for the trials performed with two volunteers (**Figure S12A**). Control experiments were carried out without the enzyme modification (**Figure S12B**) and at the same blood glucose value (fasting state).

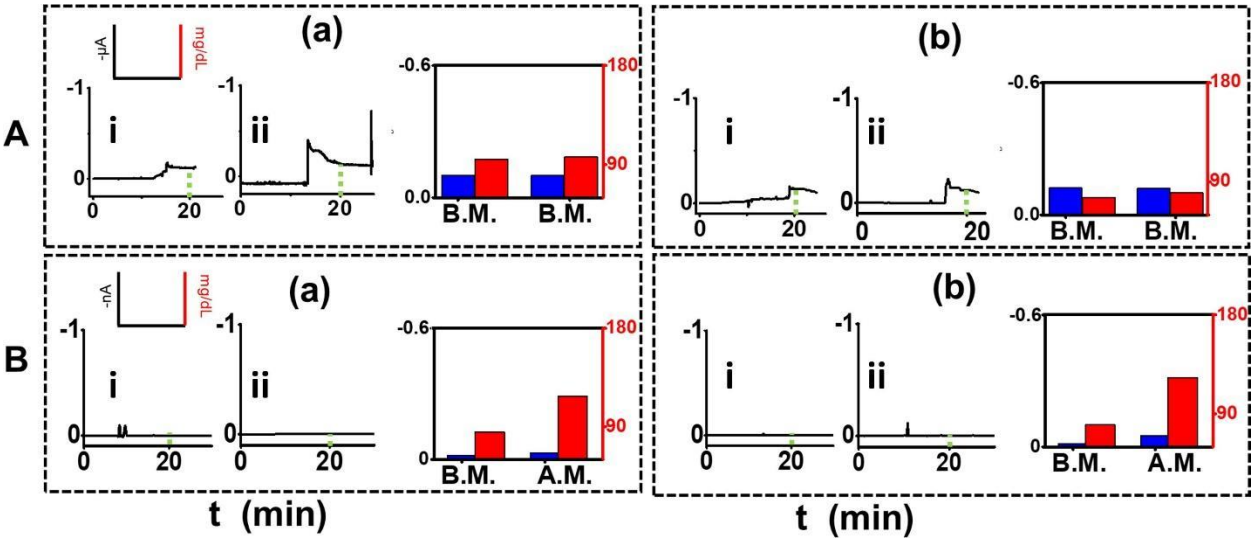


Figure S12- Control on body experiments for sweat glucose detection in stimulated sweat using the IP fluidic device A) Amperometric response at the same blood glucose value. Two different microfluidics devices (i, ii) were used for every subject (a, b). Subject sweat glucose was collected at a fasting state. Bar column graphics represent the current value (blue) obtained for every sensor and their respective blood glucose value (red). B) Chronoamperograms obtained using screen printed sensors without any enzymatic

modification. Two different microfluidics devices (i, ii) were used for every subject (a, b). Subjects sweat glucose was collected at a fasting state and after food ingestion. Bar column graphics represent the current value (blue) obtained for every sensor and their respective blood glucose value (red).

The sensor was evaluated *in-vitro*, where the selectivity of the electrochemical response to glucose in the presence of possible sweat interferents analytes (ascorbic acid, lactic acid, acetaminophen and uric acid) was studied. The results obtained during these *in-vitro* experiments showed negligible response in the presence of glucose (**Figure S13B**).

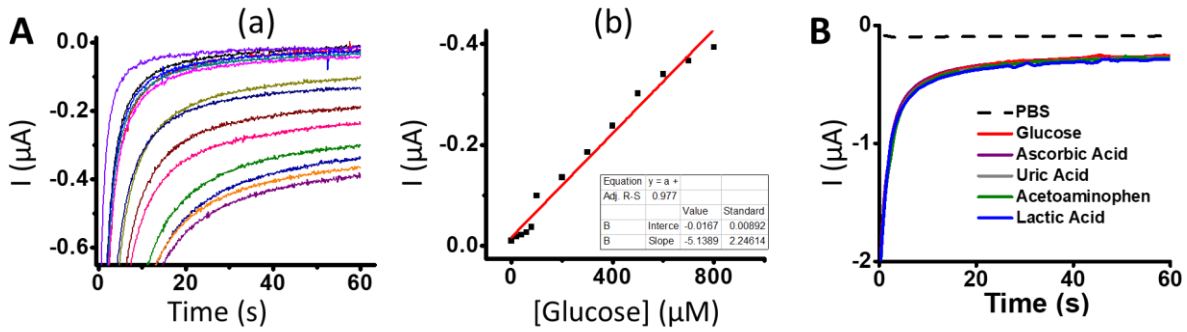


Figure S13. A) Glucose calibration. (a,b) Glucose in-vitro calibration of the GOx/PB biosensor in PBS. Calibration was taken at fixed potential at -0.2V. Low calibration range was performed from 20 – to 100 µM and high calibration range from 200 -800 µM. B) Interferent analytes. Addition of 0.1 mM glucose, 0.1 mM ascorbic acid, 10 mM lactic acid, 0.1 mM acetaminophen and 0.1 mM uric acid.