Supporting Information

Ion-Gated 3D-Printed Sensor for In Situ, Teat-Level Detection of Subclinical Mastitis

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Section S1: Reagents, Chemicals and Biomolecules

Calcium chloride ($CaCl_2$), sodium chloride (NaCl), potassium chloride (KCl), and Ag/AgCl ink (composed of finely dispersed chloritized silver flakes) were obtained from Fisher Scientific, Waltham, MA, USA. Tetrahydrofuran (THF), poly(3-octylthiophene-2,5-diyl) (POT), polyvinyl chloride (PVC), dioctyl sebacate (DOS), tetradodecyl ammonium tetrakis(4-chlorophenyl)borate (ETH 500), hydrogen ionophore I - cocktail B (Hydrogen ionophore I, 10.0 wt. %, 2-Nitrophenyl octyl ether, 89.3 wt. %, Potassium tetrakis(4-chlorophenyl) borate, 0.7 wt. %), potassium ferricyanide (III) ($K_3Fe(CN)_6$), potassium hexacyanoferrate (II) trihydrateand ($K_4[Fe(CN)_6] \cdot 3H_2O$), potassium phosphate monobasic (KH_2PO_4), potassium phosphate dibasic (K_2HPO_4), bovine serum albumin solution (BSA), N-hydroxy sulfosuccinimide sodium salt (sulfo-NHS), N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide (EDC), L-Cysteine, and Nafion were purchased from Sigma-Aldrich (St. Louis, MO, USA). Delaminated MXene ($Ti_3C_2T_x$) slurry was purchased fron Y-Carbon, Ltd., Ukraine. Single layered graphene oxide (GO) flakes were purchased from ACS materials, Pasadena, CA, USA. Deionized (DI) water ACS reagent grade, ASTM type I, having a resistivity of 18.2 MΩ.cm was purchased LabChem, PA, USA. Elastomeric dielectric ink and the curing agent purchased from Creative Materials, Ayer, MA, USA. Sylgard 184 silicone elastomer base and curing agent purchased from Dow company, Midland, MI, USA. Rabbit N-acetyl beta-D-glucosaminidase (NAGase) polyclonal antibody and N-acetyl beta-D-glucosaminidase (NAGase) recombinant protein purchased from MyBioSource, San Diego, CA, USA. The high-temperature resin cartridge (V2) was bought from Dynamism, Inc., Chicago, IL, and is used to manufacture the sensor base, device bodies, and master molds.

Section S2: Selectivity Calculation

To assess the ion selectivity of a pH sensor using the Nikolskii-Eisenman formalism, the logarithmic term in the equation can be substituted with a sum of activities modified by selectivity coefficients, as presented in the following equation.[1]

$$E = E_0 + \frac{RT}{zF} \ln(a_I + K_{IJ}^{p} a_J^{Z_I/Z_J})$$

where K_{IJ}^{p} represents the selectivity coefficient, aI and aJ stand for the activities of ions I and J in the test solution, and Z_I and Z_J denote the charges of the target and interfering ions, respectively.

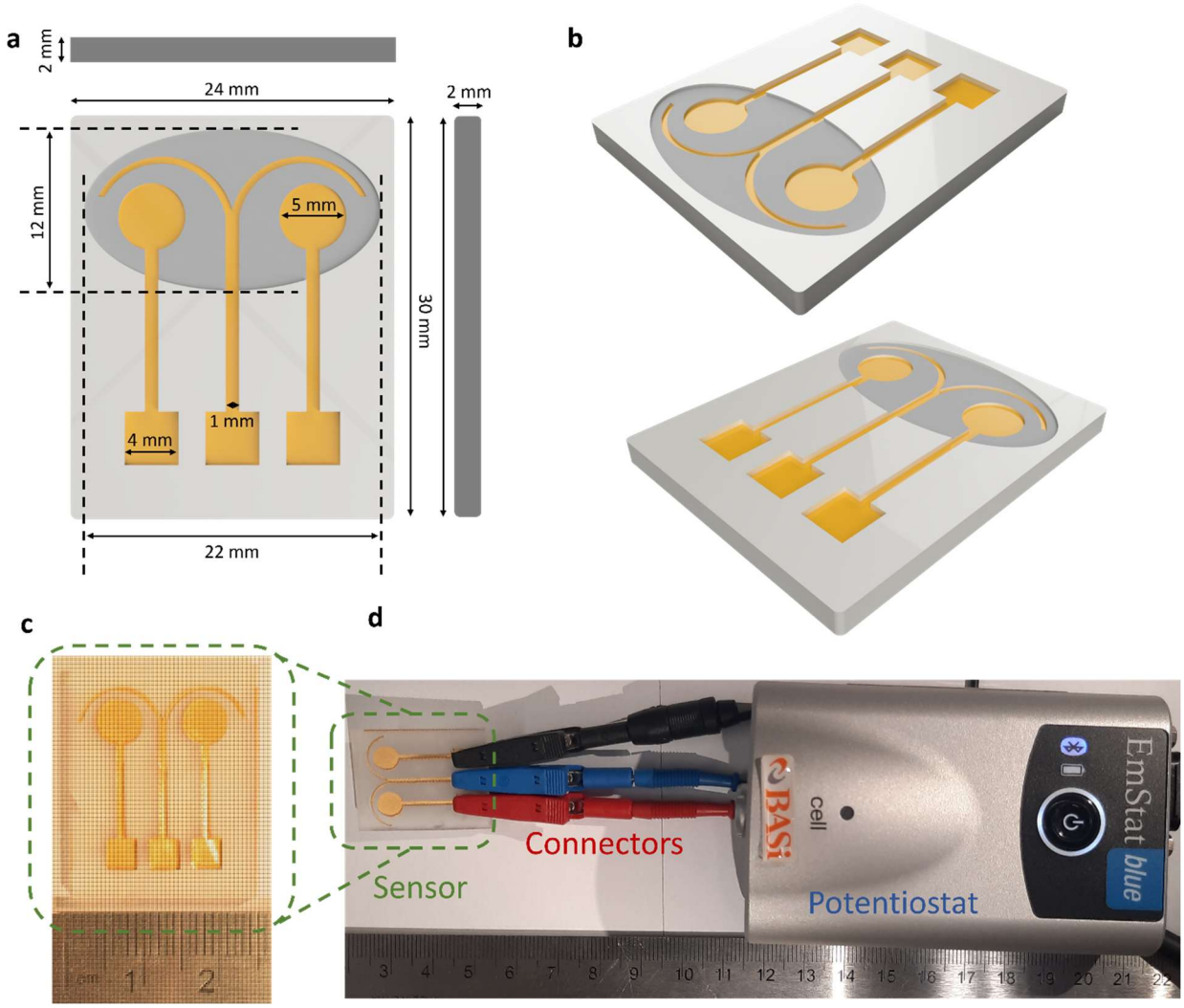

Figure S1. Details of the 3D-printed sensor. In **(a)**, front, top, and side views of the sensor with precise dimensions are presented. Each electrode has a diameter of 3 mm, and they are strategically placed on an elliptical-shaped sample holder. The Fusion 360 rendered designs in **(b)** showcase the 3D sensor from various angles. Following the printing process, **(c)** displays the sensor with gold-coated electrodes, providing dimensions. Finally, **(d)** presents an actual photograph of the device, highlighting its overall size. The sensor is seamlessly connected to a Bluetooth-enabled portable potentiostat circuit for data collection.

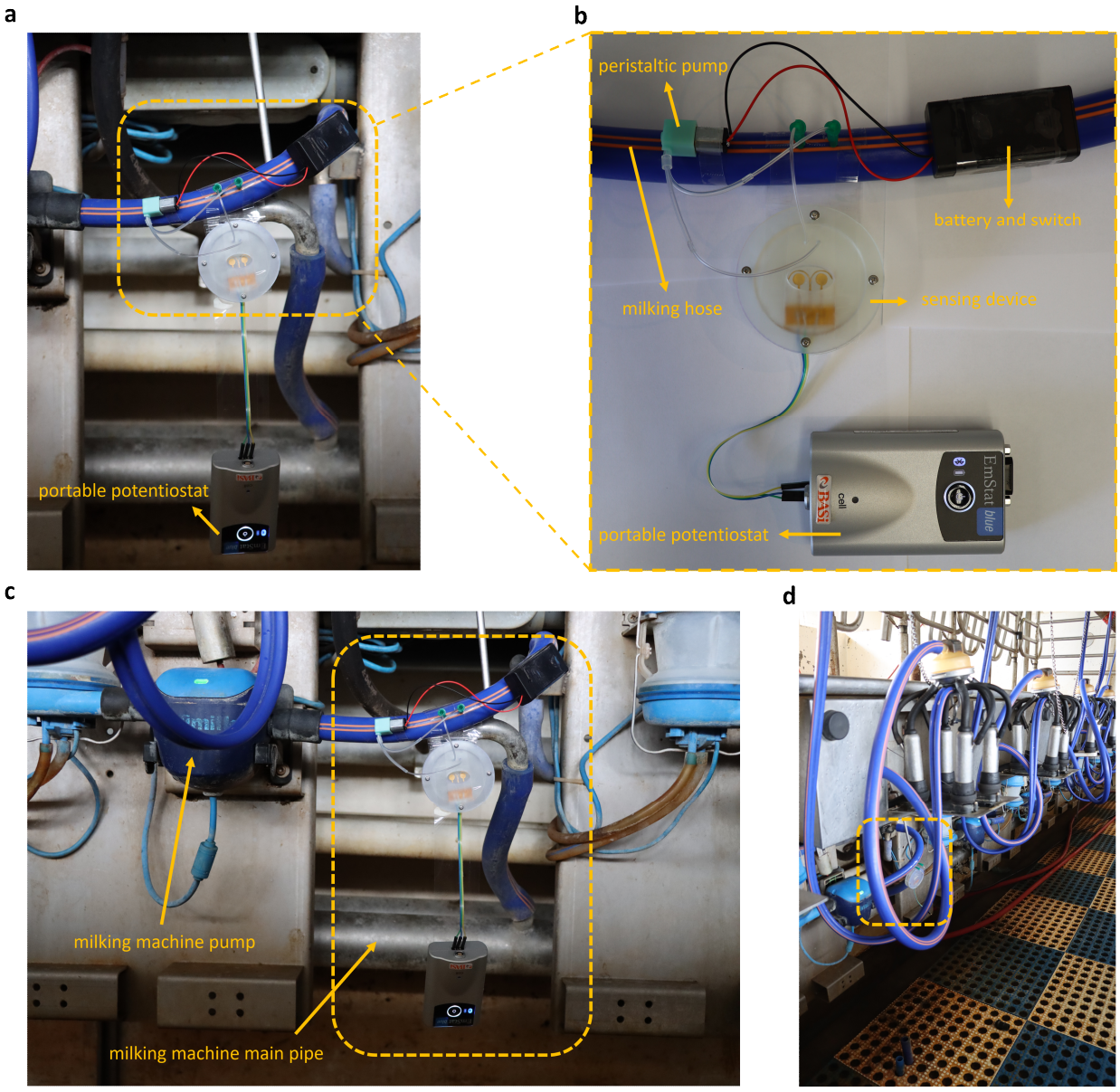

Figure S2. TeatSense demonstrates integration with an automated milking machine. (a) The TeatSense system, comprising device bodies, a PDMS microfluidic system, and a 3D-printed sensor, is integrated into the main hose of the milking machine for composite-milk monitoring. This hose transports freshly vacuumed milk from the cow positioned on the top platform into the main pipe, enabling direct sample collection from the targeted herd. The image also illustrates the integrated micro pump (300 µL/min), its power supply, and the portable potentiostat used for data collection and transfer. **(b)** The same system is displayed on the lab bench prior to integration with the milking machine, with each component clearly labeled. **(c)** A zoomed-out image depicts the integrated device alongside other components of the milking machine, including the main pump and primary pipeline. **(d)** Integration of our TeatSense system within the milking parlor.