

Comparison between Different Configurations of Reference Electrodes for an Extended-Gate Field-Effect Transistor pH Sensor

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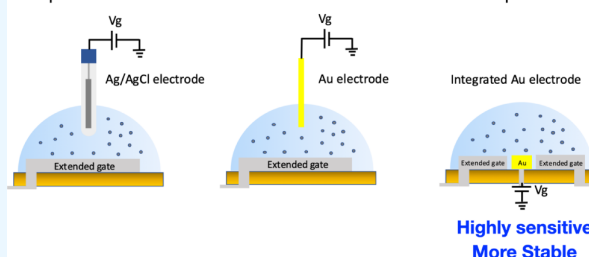
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ABSTRACT: This study investigates the performance and stability of extended-gate field-effect transistor (EGFET) pH sensors with three different reference electrode configurations: conventional external Ag/AgCl, external gold (Au), and integrated Au electrodes. The goal is to identify an optimal reference electrode configuration for enhancing the portability and manufacturability of EGFET pH sensors, which are crucial in medical, agricultural, and environmental applications. Results showed that the integrated Au electrode exhibits superior stability and comparable sensitivity to those of Ag/AgCl and external Au electrodes. The integrated Au electrode configuration achieved a 96 mV/pH sensitivity with 97% linearity across a pH range of 4 to 10. Stability tests revealed that the integrated Au electrode had the least drift under different pH conditions compared with the other two configurations, suggesting improved reliability. These findings highlight the integrated Au electrode's potential as a viable alternative to conventional reference electrodes in EGFET pH sensors, offering enhanced performance and practicality for point-of-care testing and other applications.

Comparisons between three reference electrodes for EGFET pH sensor



INTRODUCTION

Field-effect transistors (FET)-based chemical and biosensors have gained significant attention in various fields, including medicine, agriculture, and environmental surveillance due to their high sensitivity, easy scalability, and manufactural simplicity.^{1–3} The FET-based biosensor was initially invented by Bergveld (1970),⁴ who replaced the gate metal of a metal-oxide-semiconductor FET (MOSFET) with an aqueous solution to form an ion-sensitive FET (ISFET). However, the ISFET design faces challenges when measurements are needed in environments where separating wet and dry conditions is advantageous. To address this limitation, the extended-gate FET (EGFET) was developed by van der Spiegel et al.⁵

The EGFET is a derivative of the ISFET that separates the sensing domain (the extended gate) from the transistor's gate, connecting the two with a conductive wire. This configuration allows the transistor to remain in a dry environment, while the sensing domain interacts with the analyte in a wet environment. The EGFET working principle relies on changes in the surface potential of the sensing domain, which are transmitted via the wire to modulate the current through the transistor channel. This modular design enhances durability and versatility while enabling the independent optimization of the sensing domain for specific applications. Over the years, EGFETs have been adapted for detecting various analytes, including ions, proteins, and nucleic acids.⁶

A critical component of EGFETs is the reference electrode, which provides a stable reference potential for measurements. An Ag/AgCl electrode has been commonly used as a reference electrode for EGFETs due to its chemical stability and easy manufacturing.⁷ The Ag/AgCl electrode consists of a glass tube containing a chloride solution (e.g., KCl), silver wire coated with AgCl, and a porous membrane for electrical interaction.⁸ Despite its popularity and durability, the Ag/AgCl electrode has several disadvantages, particularly in the context of portable biosensors and point-of-care (POC) testing. First, the bulky configuration of traditional external reference electrodes such as the Ag/AgCl electrode impedes the miniaturization of POC devices. Miniaturization is critical for enhancing portability, reducing sample volume requirements, and enabling integration with lab-on-a-chip systems.^{7,9} For instance, miniaturized electrodes facilitate faster analysis times, lower production costs, and increased usability in a resource-limited setting.⁷ Second, the stability of reference electrodes directly influences the accuracy and reliability of biosensors. The Ag/AgCl electrode is sensitive to environmental factors

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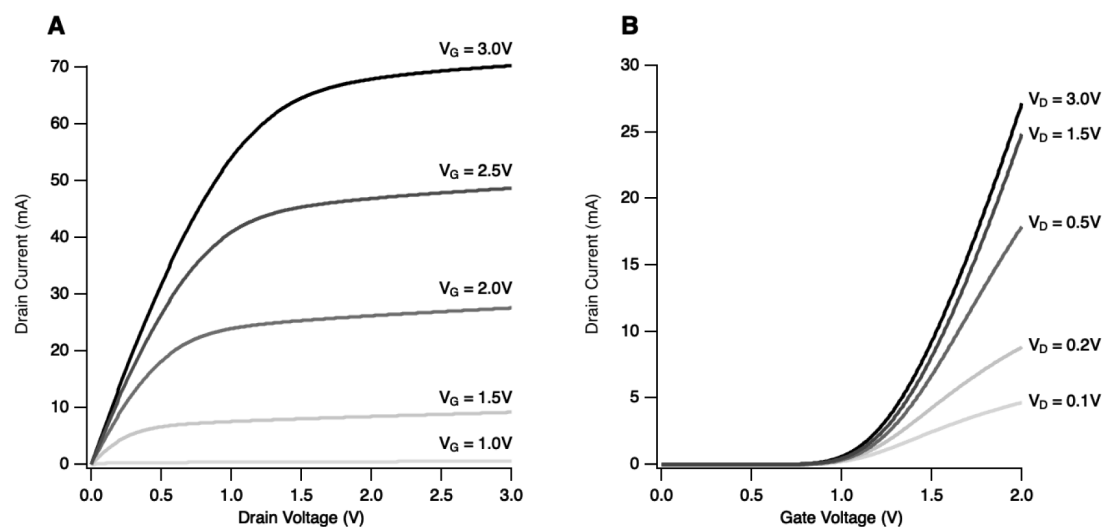


Figure 1. Transfer characteristics of the EGFET. (A) Drain current (I_D) vs drain voltage (V_D) output characteristics under different gate voltages (V_G). (B) Drain current (I_D) vs gate voltage (V_G) output characteristics under different drain voltages (V_D).

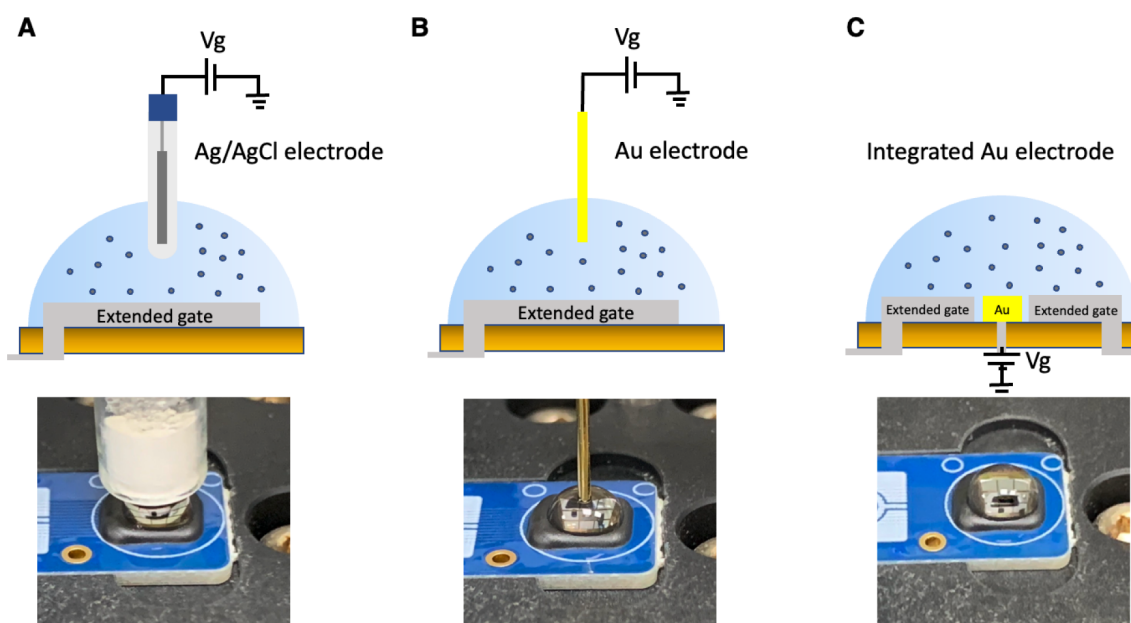


Figure 2. Configuration of EGFET pH sensor with different reference electrodes. (A) The schematic presentation (upper) and actual apparatus (lower) for the pH sensor with the Ag/AgCl reference electrode. (B) The schematic presentation (upper) and actual apparatus (lower) for the pH sensor with the external Au reference electrode. (C) Schematic presentation (upper) and actual apparatus (lower) for the pH sensor with the integrated Au electrode.

and vulnerable to electrode material degradation, resulting in potential drift and inaccuracies in measurements. Also, there's a possibility of electrolyte leakage from the Ag/AgCl electrode, which can alter the reference potential and contaminate the analyte.¹⁰ Miniaturized or integrated electrodes with well-designed diffusion barriers or solid-state alternatives have been shown to mitigate these issues, offering enhanced operational stability over time.¹⁰ Other approaches such as modifying the material (e.g., platinum reference material) for the reference electrodes or integrating the reference electrodes on the chip (e.g., ink-printed Ag/AgCl reference electrode) have been reported.^{11,12} However, studies looking into the effect of changing the material of the external reference electrode or

switching from an external reference electrode to an integrated one are lacking. In this study, we aimed to compare performance and stability of an EGFET pH sensor with three reference electrode configurations, the conventional Ag/AgCl, external Au, and integrated Au electrodes. By exploring these alternatives, we aim to advance the development of miniaturized, stable, and reliable reference electrodes that can be tailored for modern biosensor applications.

RESULTS AND DISCUSSION

We successfully constructed an N-type EGFET using the CMOS process. To characterize the electrical characteristics of the EGFET, we investigated the responses of the output drain

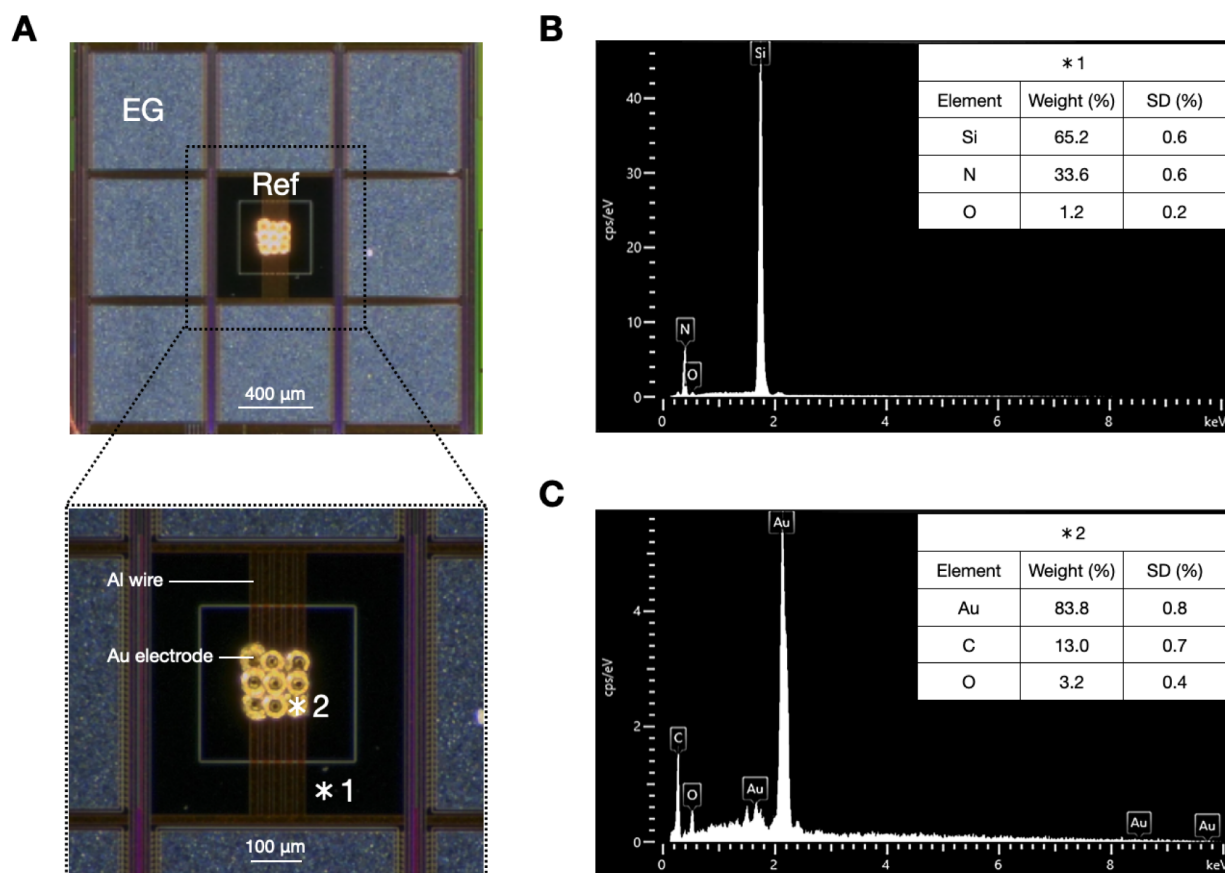


Figure 3. Material investigation of the integrated Au reference electrode. (A) Optical imaging of the EGFET with an integrated Au reference. Upper part represents the entire EGFET, with the extended gate labeled EG and region for the reference electrode labeled Ref. Dotted square represents the region which is magnified to the lower part. Lower panel shows the magnified integrated Au reference electrode. Asterisk 1 represents the location for energy dispersive X-ray spectroscopy (EDX) analysis in panel B, and asterisk 2 represents the location for EDX in panel C. (B) EDX spectrum analysis for asterisk 1 in panel A. (C) EDX spectrum analysis for asterisk 2 in panel A.

current (I_D) to a range of drain voltages (V_D), and gate voltages (V_G) (Figure 1). The behaviors of our EGFET agreed with the expectation of an N-type EGFET.

We further conjugated the EGFET with three configurations of the reference electrode, including conventional Ag/AgCl, external Au, and an integrated Au electrode, to form the pH sensing apparatus (Figure 2). Since Au is a common material for the external electrode, we first tested the impact on the pH sensing performance when changing the material for the external reference electrode from conventional Ag/AgCl to an Au electrode. There was no significant change in pH sensing response between the Ag/AgCl and Au external reference electrodes (Figure 4), indicating that Au may serve as a valid reference electrode. In the next step, we directly fabricated an Au electrode into the EGFET to form an integrated Au reference electrode after the CMOS manufacturing via Au wire-bonding (Figure 2A). Energy dispersive X-ray spectroscopy (EDX) of the region of the reference electrode showed a clear spectrum peak of Au (Figure 3B) compared with the spectrum peak of silicon nitride (Figure 3B) outside the region. The optical images (Figure 3A) and spectral analysis (Figure 3C) provided a successful fabrication of an integrated Au reference electrode. There are several advantages for an integrated Au reference electrode for the EGFET pH sensor. First, the integrated electrode is less bulky than an external

electrode (Figure 2), which exerts less perturbation to the sample solution, especially when the volume of the sample solution is small. Second is that since the integrated Au electrode can be fabricated onto the EGFET right after the CMOS process, it is more convenient and potentially more cost-effective for production. Third, an integrated reference electrode eliminates the suspending external electrode, making the ensemble more stable for hand-carrying use, such as point-of-care (POC) testing scenarios.

The pH sensing curves ranging from pH 4 to 10 for the three reference electrodes are shown in Figure 4A–C. The drain current (I_D) changes were obtained during pH sensing with a gate voltage (V_G) sweep from 0.6 to 2.0 V. The I_D – V_G function showed that the integrated Au electrode yielded the highest V_G thresholds of the three reference electrode configurations, followed by external Au and Ag/AgCl electrodes. Figure 4D–F shows the pH sensing performance for the three settings. Between pH 4, 6, 8, and 10, the sensing response showed a good linearity of 98% with a pH sensitivity of 95 mV/pH for the Ag/AgCl electrode. For the external Au electrode, the linearity of the sensing response was 88%, with a sensitivity of 112 mV/pH. Lastly, the integrated Au electrode showed a linearity of 97% and a sensitivity of 96 mV/pH.

In Figure 5A–C, we examined the stability of the three reference electrode configurations by submerging the sensing

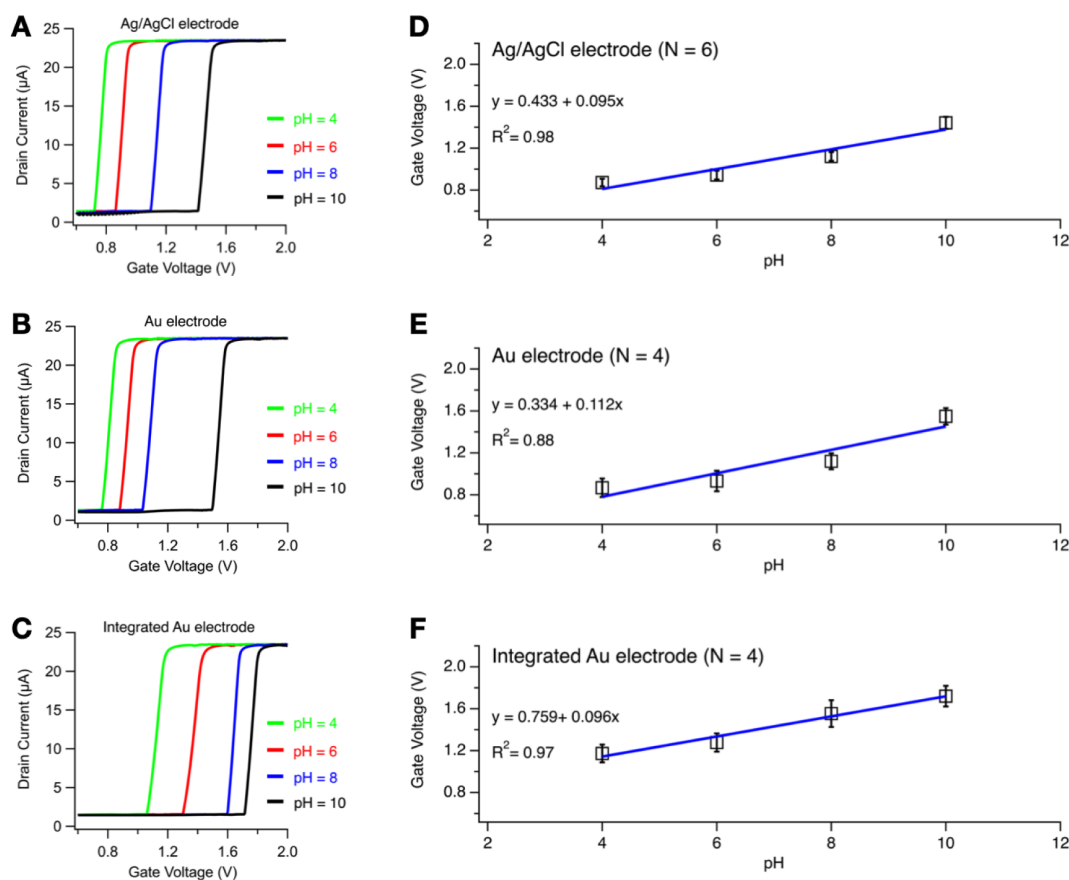


Figure 4. pH sensing characteristics and response curve of the EGFET. The responses of drain current (I_D) to gate voltage (V_G) under different pH values for Ag/AgCl reference electrode (panel A), external Au electrode (panel B), and integrated Au electrode (panel C). The response curves of gate voltage (V_G) under different pH values for Ag/AgCl reference electrode (panel D), external Au electrode (panel E), and integrated Au electrode (panel F). Square labels indicate averages with error bars indicating standard errors. Blue lines are derived from linear regression across the measurements.

apparatus in solutions of different pH values for about 16 min. We found that the stability of all configurations decreased with the increase in the solution's pH value. This may be due to the interaction between the sensing area made of Al_2O_3 and the basic solution, as shown in the 12 h submersion experiments of the extended-gate in solutions of different pH values (Figure 5D–F). The Al_2O_3 surface showed pronounced delamination and signs of erosion. The point of zero charge (PZC) for Al_2O_3 is around pH 7.5–8. Although Al_2O_3 exhibits an amphoteric behavior, which means it can react with both acids and bases, Al_2O_3 tends to have a positive surface charge in an acidic solution (i.e., pH below the PZC) and becomes more stable.¹³ Despite the decreased stability over time under a more basic solution, the stability of the integrated Au electrode was the highest across pH values (Figure 5A–C). The significant increase in sensing stability may result from the decreased perturbation for the integrated reference electrode and the increased proximity between the reference electrode and the sensing area, resulting in a more stable reference potential bias around the sensing area.

As summarized in Table 1, several groups have developed an EGFET pH sensor with an integrated reference electrode, and most of them used solid state Ag/AgCl. Our system with an integrated Au reference electrode showed better sensitivity than most of the systems with integrated reference electrodes,

indicating that Au may be a better material for constructing an integrated reference electrode. In conclusion, we fabricated three different reference electrode configurations for the EGFET pH sensor and compared their pH sensing performance. The sensing apparatus with an integrated Au reference electrode showed similar pH sensing performance and better stability when compared with Ag/AgCl and external Au electrodes. Also, the pH sensitivity for our integrated Au reference electrode was better than that of previously published systems using solid state Ag/AgCl as an integrated reference. These findings may provide insights into developing a more cost-effective and portable EGFET-based pH sensor.

METHODS

Fabrication of the EGFET. The EGFET is constructed with an N-type FET with an aluminum extended gate using a standard complementary metal-oxide-semiconductor (CMOS) procedure from United Microelectronics Corporation (Hsinchu, Taiwan). In short, the fabrication began with a silicon substrate, where a thin silicon dioxide layer was grown or deposited as the gate oxide, followed by photolithography and doping to define the source and drain regions of the N-type FET. An aluminum layer was then deposited and patterned to form the extended gate structure, which features an exposed sensing area of approximately $10,000 \mu m^2$. The entire circuit,

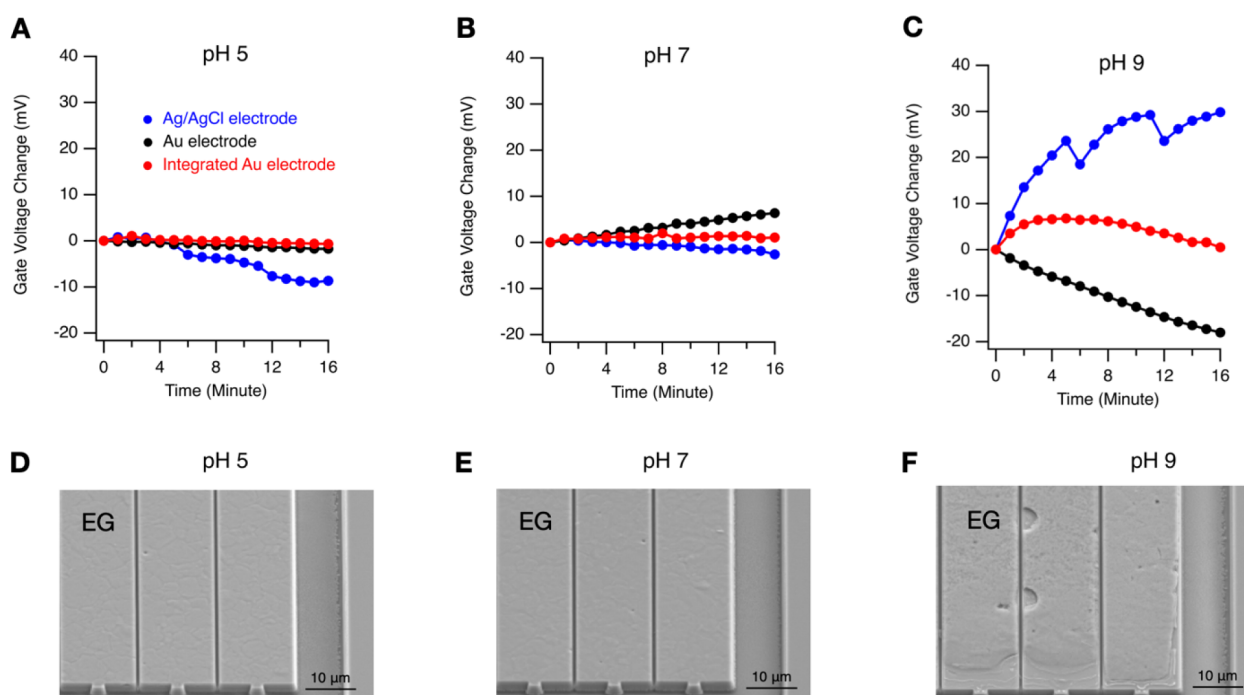


Figure 5. The stability is presented as the shift of gate voltage from baseline under pH 5 (panel A), pH 7 (panel B), and pH 9 (panel C) for different reference electrodes. The surface electron microscopy (SEM) images of the extended gate (labeled EG) after submersion in buffers with pH values of 5 (panel D), 7 (panel E), and 9 (panel F).

Table 1. Comparison between Different pH Sensing Platforms with an Integrated Reference Electrode

Reference electrode	Sensing electrode	pH sensitivity (mV/pH)	pH sensing range	Linearity (%)	Ref
Integrated solid state Ag/AgCl	ZnO	53.4	2–12	88.7	14
Metallic Ag/AgCl	ITO	52.2	2–9	98.8	15
Electroplated Ag/AgCl	ITO	84.6	4–10	99.9	16
Integrated Au	Al ₂ O ₃	96	4–10	97.0	Our study

except for the sensing area, was covered with a silicon nitride layer, which served as a robust protective dielectric. The final steps involved passivation and selective etching to expose the sensing area, followed by the formation of interconnects and electrical testing to ensure functionality.

Configuration of Different Reference Electrodes. The Ag/AgCl reference electrode (RE-1CP) was purchased from BAS Inc. (Tokyo, Japan). The electrode consisted of Ag wire coated with AgCl immersed in a saturated KCl solution. The external Au electrode (CPM-02D) was purchased from Hui-Lin Inc. (Hsinchu, Taiwan). The production of the integrated Au electrode was conducted right after the CMOS manufacturing of the EGFET. The integrated Au electrode was manufactured by an Au wire-bonding process by Ma-tek (Hsinchu, Taiwan). Au wires with a diameter of 1 mil and a purity of 99.99% (4N) were used for the wire-bonding. The bonding process was carried out using an electric-flame-off (EFO) system. This system generates a high-voltage discharge at the tail end of the Au wire, producing electric sparks that generate sufficient heat to instantly melt the tail end, forming a

free air ball (FAB) with a size of 2 mils. The EFO current was maintained at 36 mAmps to ensure consistent FAB formation. The thermomechanical bonding process parameters were optimized with a bond power of 100 mA, bond time of 80 ms, and bond force of 33 g to achieve high-quality joints. The main heat temperature during the bonding process was set to 120 °C to facilitate effective bonding while maintaining the material integrity. The bonding was performed using a tip size of 8 mil and a capillary velocity rate of 1 mil/ms.

Material Investigation of the EGFET and Integrated Au Electrode. Optical microscopy imaging of the EGFET with the integrated Au electrode was performed using a customized optical microscope (model ET2026-240129) from Chain-Logic International Corp. (Hsinchu, Taiwan). Elemental analysis of the integrated Au electrode was conducted using the built-in energy dispersive X-ray spectroscopy (EDX) system integrated within a scanning electron microscope (SEM; model Rugulus 8220, Hitachi, Japan) according to the manufacturer's protocol. In brief, samples were cleaned, mounted on aluminum stubs, and sputter-coated with gold for conductivity. The SEM was operated at an acceleration voltage of 10 kV. Spectral data from selected regions were analyzed using EDX software to identify and quantify elemental composition. This analysis enabled the identification and quantification of the elemental composition, ensuring the purity and proper integration of gold in the electrode.

Dry Test. The transfer characteristics of the EGFET, including the ID-Vd and ID-Vg curves, were profiled using a dual-channel sourcemeter (Keithley 2636, Keithley Instruments, Ohio, U.S.). The ID-Vd measurement involves sweeping the drain voltage (Vd) from 0 to 3 V, while the gate voltage is changed sequentially to 1.0, 1.5, 2.0, 2.5, and 3.0 V. The ID-Vg measurement involves sweeping the gate voltage

(V_g) from 0 to 2 V, while the drain voltage is changed sequentially to 0.1, 0.2, 0.5, 1.5, and 3.0 V.

pH Sensing. Bis-tris propane (BTP) buffer with a concentration of 10 mM was used for pH sensing. The pH of the buffer was adjusted to the desired pH using HCl and NaOH. For pH sensing using external reference electrodes (Ag/AgCl and Au), 20 μ L of the BTP buffer was added to the surface of the sensor, followed by the placement of the external electrodes on top of the solution (Figure 1). For pH sensing using the chip with an integrated Au reference electrode, 20 μ L of the BTP buffer was directly added to the sensing surface. The V_D – V_G curve was obtained by a customized reader measuring the V_D under a V_G sweep between 0.6 and 2 V. Once the measurement was completed, the solution was removed, and the surface was washed with BTP buffer before switching to the buffer with different pH values.

Stability Test. For the stability test of different reference electrodes, the EGFET with three configurations (Ag/AgCl, external Au, and integrated Au) was submerged in buffer solutions at pH 5, 7, and 9 for 16 min. Gate voltage changes were measured every minute to monitor the stability of the reference electrode configuration during the sensing process. For the stability test of the extended gate, the EGFET was submerged in the same buffer solutions (pH 5, 7, and 9) for 12 h. After the extended immersion, surface imaging of the extended gate was performed using a scanning electron microscope (SEM; model Rugulus 8220, Hitachi, Japan) to assess potential material degradation or morphological changes caused by prolonged exposure to the buffer solutions according to the manufacturer's protocol. In brief, samples were cleaned, dried, and mounted on aluminum stubs with conductive adhesive, followed by sputter-coating with a thin layer of gold to enhance conductivity. Imaging was conducted under an acceleration voltage of 10 kV in high vacuum mode. A secondary electron detector was used to capture the surface morphology, and adjustments to focus, contrast, and brightness were made to ensure high-resolution image quality.

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Conceptualization: J.T.Y. and C.F.K. Data curation: C.Y.H. and H.W.L. Formal analysis: J.T.Y. and C.Y.H. Funding acquisition: C.F.K. Investigation: J.T.Y., C.Y.H., H.W.L., and C.F.K. Methodology: C.Y.H., H.W.L., and C.F.K. Project administration: J.T.Y. Resources: C.Y.H., H.W.L., and C.F.K. Software: J.T.Y., C.Y.H., and H.W.L. Supervision: C.F.K. Validation: H.W.L. and C.F.K. Visualization: J.T.Y. and C.Y.H. Writing—original draft: J.T.Y. and C.F.K. Writing—review and editing: J.T.Y., C.Y.H., H.W.L., and C.F.K.

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Notes

The authors declare no competing financial interest.

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