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Construction and optimization of a versatile bioFET platform for detecting nucleic acids and protein using the biotin–neutravidin affinity system

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ABSTRACT

A biosensor based on a field-effect transistor (bioFET) is a promising system for point-of-care testing due to its portability and high sensitivity. A bioFET uses a linker platform to connect capture molecules to the FET, which detects electrical changes when targets bind to the capture molecules. This study presents the development and optimization of a versatile, highly sensitive bioFET platform utilizing a biotin–neutravidin affinity system capable of detecting both DNA and proteins. The bioFET was constructed using an extended-gate field-effect transistor (EGFET) with an aluminum gate functionalized with (3-aminopropyl)triethoxysilane (APTES)–biotin–neutravidin. We optimized the concentrations and molar ratio of biotin and neutravidin using fluorescence imaging and biosensing benchmarks. We found that 0.1 μg/mL biotin paired with 30 μg/mL neutravidin, approximately 1:1 in molarity, achieved optimal sensitivity. The optimized bioFET system demonstrated good sensitivity, with lower detection limits of 3.5 copies for *Escherichia coli* genomic DNA and 0.3 fg/mL for p-Tau217, an Alzheimer's disease biomarker. In summary, this study establishes the ideal conditions for constructing a bioFET system using an APTES–biotin–neutravidin linker capable of detecting DNA and proteins, with potential for convenient and sensitive point-of-care diagnostics.

1. Introduction

With the emergence of novel infectious diseases and the advancement of precision medicine, there is an increasing need for point-of-care tests (POCT) that can rapidly and accurately detect disease-related biomarkers. Among biosensing platforms, field-effect transistor (FET) biosensors (bioFET) are leading candidates for POCT due to their portability, sensitivity, and ease of manufacturing [3,9]. The basic components of a bioFET include a source electrode and a drain electrode connected through a semiconductor channel, with a separate gate electrode whose voltage (V_g) modulates the current flowing between the source and drain electrodes based on the voltage difference between the drain and source electrodes (V_d). When charged biomolecules bind to the sensing surface on the gate electrode, they induce a change in the surface potential, altering the gate voltage (V_g) required to maintain a given drain current (I_d) [10].

Because the bioFET detection mechanism measures the change in current after target molecules bind to the capture system on the gate surface, the design of the sensing surface and capture system is crucial for bioFET performance. The most common strategy for functionalizing the metallic gate surface involves silanization with bifunctional silanes, such as (3-aminopropyl)triethoxysilane (APTES) [11]. After functionalization, probes for target molecules can be immobilized on the surface using linkers. A common linker for conjugating probes to an APTES-functionalized surface is the biotin–avidin linkage system. The interaction between biotin and avidin is among the strongest non-covalent bonds in nature, with a dissociation constant (K_d) of approximately 10^{-15} M; thus, biotin–avidin systems are widely used as probes or affinity systems [5]. Biotin, a small molecule consisting of a tetrahydrothiophene ring fused to a tetrahydroimidizalone ring, can be conjugated to APTES after modification with N-hydroxysuccinimide ester (NHS) [6,8]. Avidin, a tetrameric protein with four biotin-binding sites, acts as a bidirectional linker, with one end attached to the APTES–biotin surface and the other linking biotinylated molecules [4].

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APTES–biotin–avidin linker systems serve as versatile platforms for conjugating various probes, including biotinylated DNA/RNA and biotinylated antibodies. Although these systems have been used in several biosensor platforms, the optimal concentrations and proportions of biotin and avidin for the APTES–biotin–avidin linker system in a bioFET remain unclear.

In this study, we constructed and optimized a versatile, highly sensitive bioFET system using our in-house extended-gate field-effect transistor (EGFET) platform. Unlike a conventional FET, an EGFET exhibits a physically separated sensing domain (referred to as the extended gate) connected to the gate via a metallic wire, enabling the separation of wet (biological material) and dry (chips) environments. Our EGFET platform was fabricated using the standard complementary metal-oxide-semiconductor (CMOS) process with an N-channel FET and an aluminum extended gate. We then conjugated the APTES–biotin–neutravidin linker system to the extended gate. Neutravidin, a deglycosylated version of chicken avidin, displays fewer non-specific interactions than avidin [7]. Sensing performance with varying proportions of biotin and neutravidin was evaluated using fluorescence imaging. Under optimal conditions, the bioFET system demonstrated high sensitivity for both DNA and protein targets. For DNA sensing, the lower limit of detection (LOD) was approximately 3.5 copies for *Escherichia coli* genomic DNA, a common pathogen in sepsis patients. The same linkage configuration produced a sensitivity of 0.3 fg/mL for tau protein phosphorylated at threonine 217 (p-Tau217), a key biomarker for Alzheimer's disease [2].

2. Materials and methods

2.1. Fabrication of the EGFET

The EGFET was constructed using an N-channel FET with an aluminum extended gated with the length and width of the channel between source and drain being 0.65 μm and 0.5 μm, respectively. The entire circuit was covered by oxide layer except for the reaction area of the extended gate. The length and width of the exposed extended gate is 650 μm and 15 μm, constructing a sensing area of 10,000 μm^2. The EGFET was fabricated using standard CMOS procedures at United Microelectronics (Hsinchu, Taiwan). An external Au electrode (CPM—02D, Hui-Lin inc, Hsinchu, Taiwan was used as the reference electrode.

2.2. Chip cleaning and surface modification

The chips were washed with acetone (Echo Chemical, Miaoli, Taiwan) and 99.5 % ethanol (Echo Chemical) to remove unwanted chemical compounds through sonication for 10 min, followed by heating in an oven at 120 °C to eliminate excess ethanol. The chips were then treated with oxygen plasma for 1 min using a plasma cleaner (PDC-00; Harrick Plasma, NY, US) to form OH terminals on the chip surface. For surface modification, the chips were soaked in 2 % APTES (Sigma-Aldrich, MO, US; diluted in 99.8 % ethanol) for 30 min to introduce amine groups on the surface. The chips were subsequently washed with 99.5 % ethanol through sonication for 10 min and then dried in an oven.

2.3. Biotinylation of the APTES-functionalized surface

NHS-biotin (Thermo Scientific, MA, US) stock solution (10 μg/μL in DMSO) was diluted to a working solution using 10 mM Bis-Tris propane (BTP) buffer (Sigma-Aldrich). To biotinylate the APTES-functionalized surface, 100 μL of biotin solution was applied to the sample well and the chip surface was submerged for 16 h at 4 °C. The biotin solution was then removed, and the chip was rinsed five times with 70 μL of wash buffer [10 mM BTP with 0.05 % Tween-20 (Sigma-Aldrich)] followed by five rinses with 70 μL of 10 mM BTP buffer.

2.4. Fluorescence imaging of biotinylation of the APTES-functionalized surface

In total, 100 μL of fluorescent reagent (biotin-DyLight488; Thermo Scientific) were added to the well. The chip was placed in a dark box to avoid light exposure and incubated at 4 °C overnight. After incubation, the reagent was removed, and 5 μL of mounting medium (S3023; Dako North America, CA, US) were applied to the chip surface. The chip was covered with a coverslip for fluorescence imaging using a confocal fluorescence microscope (TCS SP8X; Leica Microsystems, Wetzlar, Germany).

2.5. Neutravidin conjugation

Neutravidin (Thermo Scientific) stock solution (3 mg/mL in 10 mM BTP buffer) was diluted to the target concentration using 10 mM BTP buffer. Seventy microliters of the diluted neutravidin solution were applied to the sample well, and the chip surface was submerged for 10 min at room temperature. After submersion, the neutravidin solution was removed, and the chip surface was washed five times with 70 μL of wash buffer, followed by five washes with 70 μL of 10 mM BTP buffer.

2.6. Preparation and conjugation of biotinylated probes for E. coli genomic DNA and biotinylated antibody for p-Tau217

To detect *E. coli* genomic DNA, primers and probes with the following sequences were synthesized by Sigma-Aldrich based on a previous study [12]**:** forward primer, biotin-GGCGAGAAACTGGC GATCCTTA; probe: biotin-TTTGGTACGCTGATGCCAGACG; and reverse primer, biotin-CGCTTCATCAAGCGGTTTCACA.

The p-Tau217 antibody was developed in-house. Female BALB/c mice (6–8 weeks old; National Laboratory Animal Center, Taipei, Taiwan) were immunized with the p-Tau217 peptide. After immunization, the spleens of these mice were collected and fused with myeloma cells for hybridoma preparation and semi-solid selection (ClonaCell Hybridoma Kit, STEMCELL Technologies, Vancouver, Canada). High-affinity antibodies produced by the hybridoma were selected using protein G Sepharose resin (Cytiva, MA, US) and concentrated with Amicon Ultra-15 centrifugal filter units (Merck Millipore, MA, US). The p-Tau217 antibody was biotinylated with EZ-Link Sulfo-NHS-Biotin (Thermo Scientific), in accordance with the manufacturer's protocol. Briefly, 5 mg/mL of purified p-Tau217 monoclonal antibody were dissolved in PBS. Biotin reagent solution was added to the antibody solution to a final concentration of 10 mM, with a 30-min incubation at room temperature. The labeled p-Tau217 monoclonal antibody was then purified for optimal performance and stability using desalting columns (Zeba Spin Desalting Columns; Sigma-Aldrich).

To conjugate the biotinylated probes to the APTES-biotin-neutravidin system, 70 μL of the biotinylated p-Tau217 antibody [1 μg/mL in PBS (Sigma-Aldrich)] or *E. coli* probes (10 μg/mL in ddH_2O) were added to the sample well, and the chip surface was submerged for 10 min at room temperature. The probe solution was then removed, and the chip surface was washed five times with 70 μL of wash buffer, followed by five washes with 70 μL of 10 mM BTP buffer.

2.7. Sample preparation

E. coli genomic DNA was kindly provided by Dr. Huang Chung-Guei at Chang Gung Memorial Hospital, Taiwan. The p-Tau217 peptide was synthesized by BIOTOOLS (New Taipei City, Taiwan).

2.8. Biosensing procedure

To ensure the reliability of the measurement under the stable drop environment, current passing through the 10 mM BTP buffer was monitored and only chips with leak current <100 nA would be accepted

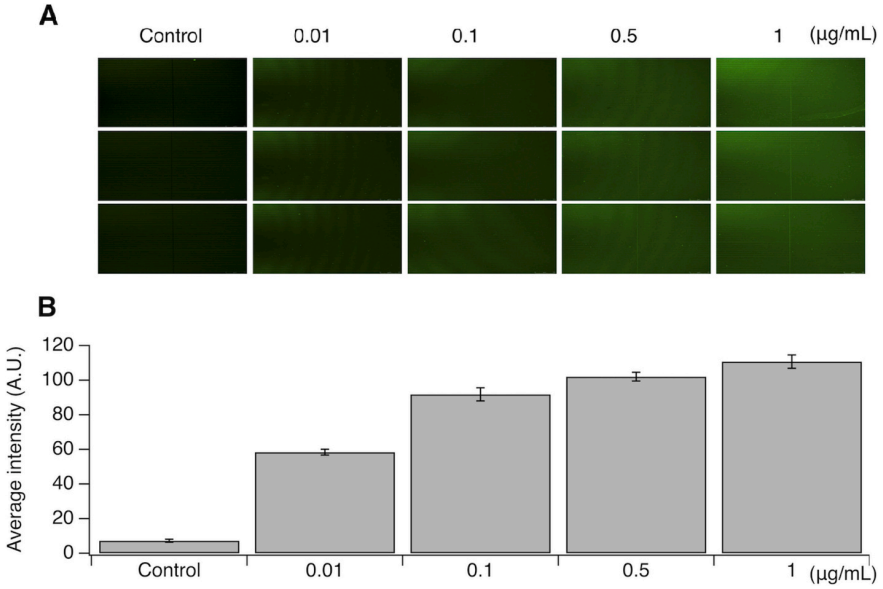

Fig. 1. Fluorescence intensity on the extended gate with different biotin concentrations. (A) Confocal microscopy images from three replicate experiments at different concentrations. (B) Quantification of average fluorescence intensities. Bars represent means, and error bars indicate standard deviations from three independent experiments. All groups were significantly different from each other.

for further measurement. The electric signals of the bioFET at different sample concentrations were obtained using a customized reader that measured the drain voltage (V_d) and performed a gate voltage (V_g) sweep from 0 to 2 V while the drain current (I_d) was set at 150 μA. To establish the baseline, 70 μL of 10 mM BTP buffer were loaded in the sample well. The $V_d - V_g$ curve was obtained after a 10-min wait to allow the system to stabilize. After the baseline measurement, the buffer was removed, and the chip was rinsed five times with 70 μL of 10 mM BTP buffer. Samples with different concentrations were then loaded sequentially using the same procedure to obtain the signal. To quantify sample concentration effects, gate voltage shifts were measured at a fixed drain voltage of $V_d = 1$ V. This value was chosen as it lies at the midpoint of the linear region in the $V_d - V_g$ response curve, thereby facilitating reliable and reproducible detection of shifts induced by different sample concentrations.

2.9. Statistics and data analysis

All continuous data are presented as the mean ± standard deviation. Data were obtained from three replicates of each experiment. Statistical analysis and visualization were performed using Igor 7 software (WaveMetrics, OR, US). Multiple comparisons were made using analysis of variance followed by Tukey's test, with the significance threshold regarded as 0.05. LODs were calculated using residuals from the linear regression plus three times the standard deviation of the residuals.

3. Results and discussion

To determine the optimal concentration of biotinylation on the APTES-modified surface, the surface was incubated with biotin at concentrations ranging from 0.01 to 1 μg/mL. A fluorophore-tagged biotin antibody was used to detect the overall fluorescence intensity as a proxy for the robustness of biotinylation. As shown in Fig. 1A, in all three replicate experiments, fluorescence intensities gradually increased with biotin concentration, peaking at 1 μg/mL. The average fluorescence intensity was significantly higher at 1 μg/mL of biotin than in all other conditions (Fig. 1B).

The performance of the linkage system was determined by the biosensor's sensitivity, which is reflected in the voltage shift of the bioFET

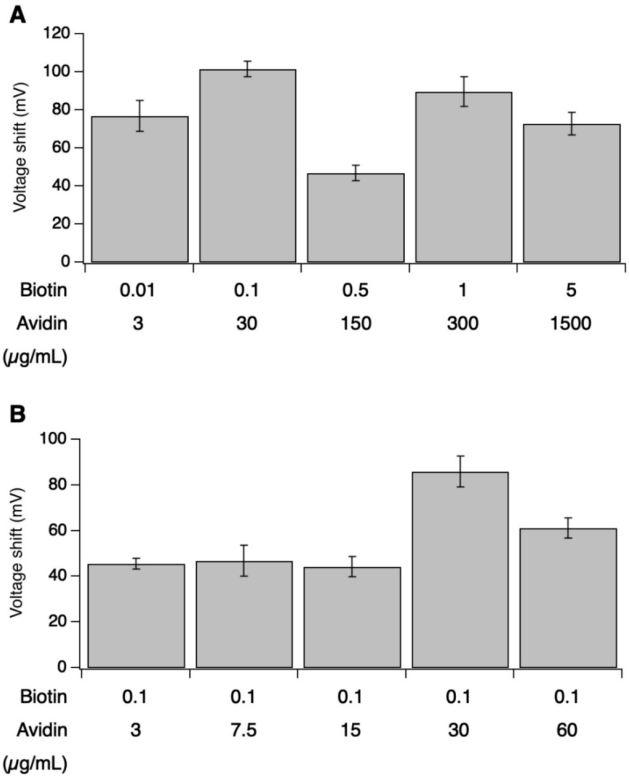

Fig. 2. Voltage shifts during *E. coli* genomic DNA sensing with different concentrations of biotin–neutravidin. (A) Voltage shifts with different biotin–neutravidin concentrations under a 1:1 molarity ratio. (B) Voltage shifts with different molarity ratios between biotin and neutravidin. Bars represent means, and error bars indicate standard deviations from three independent experiments. Different letters above the bars indicate statistical significance.

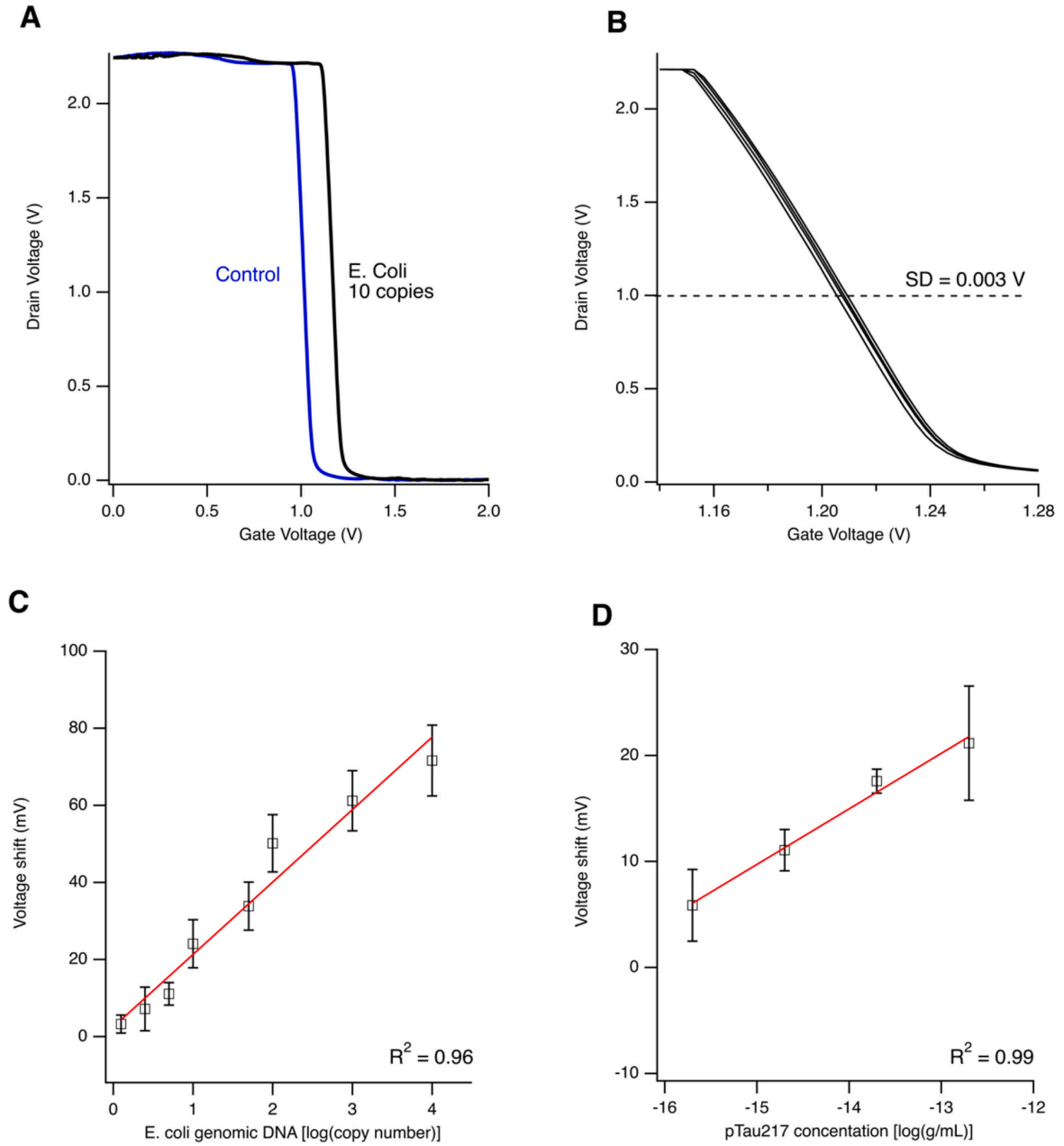

Fig. 3. Biosensing performances for *E. coli* genomic DNA (left) and p-Tau217 protein (right). Red lines represent linear regression lines, and error bars indicate standard deviations from three independent replicates. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

when the biological target binds to the probe. Thus, we measured the voltage shift of the bioFET upon detection of 10^3 copies of *E. coli* genomic DNA with different concentrations of biotin and neutravidin (Fig. 2). Based on the finding that incubation with 1 μg/mL biotin resulted in the highest biotinylation on the APTES-treated surface (Fig. 1), we hypothesized that 1 μg/mL of biotin would be the optimal concentration for subsequent neutravidin conjugation. In Fig. 2A, we began with a fixed 1:1 molarity ratio between biotin (molecular weight 244.31 g/mol) and neutravidin (molecular weight 60,000 g/mol), which was approximately 1:300 in mass/volume concentration, and compared the voltage shifts for different biotin–neutravidin concentration pairs. Surprisingly, across the wide concentration range tested, the combinations of 0.1 μg/mL biotin with 30 μg/mL neutravidin and 1 μg/mL biotin with 300 μg/mL neutravidin exhibited the largest voltage shifts, indicating the greatest potential sensitivities. Although there was no significant difference in the voltage shifts between 0.1 and 1 μg/mL biotin, we chose 0.1 μg/mL biotin for further optimization due to the numerically higher voltage shift and more efficient material utilization. Aisyiyah Jenie et al. [1] also reported that high biotin or neutravidin concentrations may decrease the binding efficiency due to overcrowding of the binding sites.

Because neutravidin has four biotin-binding sites, we hypothesized that the molarity ratio between neutravidin and biotin influences conjugation of the capture molecule (biotinylated DNA or antibody) [4]. Therefore, we tested the sensitivity of the apparatus using 0.1 μg/mL biotin with neutravidin molarity ratios ranging from 1:0.1 (0.1 μg/mL

biotin with 3 μg/mL neutravidin) to 1:2 (0.1 μg/mL biotin with 60 μg/mL neutravidin) (Fig. 2B). The 1:1 molarity ratio, consisting of 0.1 μg/mL biotin and 30 μg/mL neutravidin, still produced the highest voltage shift.

Based on the biotin and neutravidin concentrations tested, we found that 0.1 μg/mL biotin and 30 μg/mL neutravidin was the optimal condition as a linkage system for the APTES-treated surface. We conjugated a biotinylated DNA probe to *E. coli* genomic DNA and a biotinylated antibody to p-Tau217 separately on the linkage system to assess the biosensing performance. The $V_d - V_g$ curves for biosensing were shown in Fig. 3A, demonstrating a voltage shift upon the detection of *E. coli* DNA. The measurement of our system was reliable and stable. Fig. 3B showed an example with four repeated measurements under 10 copies of *E. coli* DNA. The overall standard deviation (SD) of V_g when $V_d = 1$ V was 0.003 V. The bioFET demonstrated good sensing performance for both DNA (Fig. 3C) and protein targets (Fig. 3D), with linearities of 0.96 (DNA) and 0.99 (protein), and an approximately 10,000-fold dynamic range in concentration. The estimated LODs were 3.5 copies for *E. coli* genomic DNA and 0.3 fg/mL for p-Tau217. Our bioFET system has several advantages. For DNA detection, it does not require DNA amplification via polymerase chain reaction. For protein detection, the sensitivity of our system surpasses that of conventional enzyme-linked immunosorbent assays, which are typically around the pg/mL level. Our study provides a novel contribution by optimizing a biotin–neutravidin linker system that achieved unprecedented sensitivity for detecting both nucleic acids and protein biomarkers. Previous studies have typically employed higher concentrations and have not systematically demonstrated the universal applicability of the same optimized conditions for both DNA and protein detection with such low detection limits. Thus, this optimized bioFET platform represents a versatile and highly sensitive tool, beneficial for developing next-generation point-of-care diagnostics.

4. Conclusion

In this study, we developed a bioFET system using EGFET and an APTES–biotin–neutravidin linker. After we optimized the concentrations and ratio of biotin and neutravidin, the system was capable of detecting biological targets, including DNA and protein, with excellent sensitivity.

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CRediT authorship contribution statement

Jiunn-Tyng Yeh: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Project administration, Investigation, Formal analysis, Conceptualization. **Lian-Chin Wang:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Chi-Pei Weng:** Writing – review & editing, Resources, Methodology, Investigation, Data curation. **Chang-Fu Kuo:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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