<Research Article>

Novel simple bacteria detection system combining signal accumulation type of ion sensitive field effect transistor (SA-ISFET) and immunoassay

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Summary A novel semiconductor ion sensor system for bacterial detection was developed by combining the signal accumulation type of an ion-sensitive field-effect transistor (SA-ISFET) with immunoassay technique. Primary antibody against *Escherichia coli* and horseradish peroxidase (HRP)-conjugated secondary antibody were used. After removing the antibody complexes bound to the target bacteria, the activity of unbound HRP conjugated to secondary antibodies was measured using SA-ISFET. The SA-ISFET amplifies the output by repeatedly accumulating the measurement signal, enabling high-sensitivity detection of even slight changed in enzyme activity. As a result, the presence of *E. coli* at concentrations of 5.0×10^5 cfu/mL or higher was successfully detected by measuring HRP enzymatic activity as the sensor output. Subsequently, an antibody with broad specificity that exhibited significant cross-reactivity with 10 frequently isolated bacterial species from environmental samples was identified. Using this low-specificity antibody with broad reactivity, it was demonstrated that multiple bacterial species could be detected using the SA-ISFET system.

Key words: Signal accumulation type of ion sensitive field effect transistor (SA-ISFET), Sensor, Immunoassay, Antibody, Bacteria

1. Introduction

In recent years, there has been a rapidly increasing demand in the fields of biotechnology and life sciences for highly sensitive and rapid sensing technologies applicable to various areas, including disease diagnosis, drug discovery, food safety assessment, and environmental monitoring[1]. Among these, sensor technologies capable of real-time and label-free detection of subtle changes associated with biomolecular and cellular responses have attracted considerable attention, with promising applications in clinical diagnostics and on-site monitoring[2].

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Although current biosensors typically rely on current detection mechanisms, semiconductor-based sensors that can detect electrical potentials are also available. Semiconductor ion sensors that measure the proton concentration (hydrogen ion concentration, pH) of sample solutions can be constructed using ion-sensitive field-effect transistors (ISFETs).

An ISFET controls the current between the source and drain by applying a voltage to the reference electrode. When a sample solution contacts the ion-sensitive film (SiO_2, Si_3N_4, Ta_2O_5, etc.) on the gate, an interface potential is generated based on the ion activity in the solution. By forming a sensing layer responsive to ions on the gate-insulating membrane, the ISFET detects the ion concentration through the change in the channel potential induced by surface potential variations (Fig. 1a)[3]. For the pH sensors, the interface potential is recorded as a pH-dependent output voltage.

Furthermore, coating the gate region with various ion-sensitive membranes enables the fabrication of diverse potential detecting sensors. Biosensors using ISFETs are manufactured using integrated circuit fabrication processes that allow miniaturization, standardization, and cost reduction through mass production. ISFETs integrated with neural networks and machine learning have also been developed to improve the measurement accuracy, showing promising applications in the food and medical industries4. However, despite their use in pH measurements, ISFETs suffer from low sensitivity and unstable outputs over time, which limit their accuracy in detecting ion concentrations when used alone. Consequently, ISFET-based biosensors have not yet gained widespread adoption compared to current biosensors.

To address these limitations, a promising alternative is the signal accumulation type of ion sensitive field effect transistor (SA-ISFET). The SA-ISFET is an improved version of the conventional ISFET based on complementary metal-oxide semiconductor (CMOS) technology, which allows electric charge to

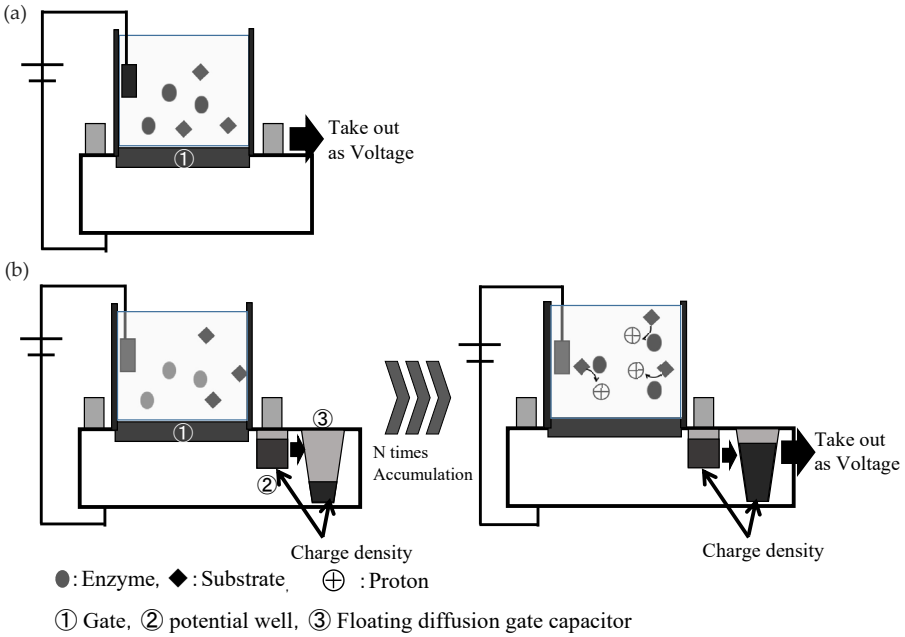

Fig. 1. Schematic illustrations of (a) a conventional ISFET system and (b) a signal-accumulating ISFET (SA-ISFET) system. (a) In the conventional ISFET system, the sample solution is applied directly to the sensor surface, and changes in proton concentration are detected in real time as a voltage signal. (b) In the SA-ISFET system, the sample is introduced to the sensor after a reset operation. Proton fluctuations in the solution are stored in a potential well between the gates, and the accumulated charge in the floating diffusion region is subsequently measured as a potential change. The output is expressed in millivolts, enabling time-integrated detection of proton activity.

be stored within the sensor. This design enables repeated measurements to accumulate signals within the device, which are subsequently amplified and output as an electrical signal[5].

As illustrated in Fig. 1b, the device detected changes in the interfacial potential caused by variations in the proton concentration before and after the enzyme reactions via an ion-sensitive membrane. The corresponding charge was stored in the internal capacitor of the sensor. By utilizing a CMOS-based structure, the accumulated charge can be incrementally integrated over multiple measurements and read as a voltage signal. This accumulation and readout mechanism enhances the signal-to-noise ratio (S/N ratio), allowing the device to detect subtle changes in the ion concentration with high sensitivity and precision. Consequently, the SA-ISFET functioned as a highly sensitive and reproducible sensor device. Previous studies have established activity measurement systems using specific enzymes such as urease and horseradish peroxidase (HRP), demonstrating the fundamental utility of SA-ISFETs[6-11]. Based on these findings, we explored further applications of this sensor technology, focusing on its practical use in microbial detection.

In this study, we developed a novel bacterial detection method that combined an SA-ISFET sensor with an antigen-antibody reaction. Reports of ISFET use in bacterial detection are extremely limited[12], and the conventional sandwich enzyme-linked immuno-sorbent assay (ELISA) requires multiple complex steps, such as immobilization and washing. In contrast, SA-ISFET provides a new, simple, and rapid detection approach that eliminates these cumbersome procedures.

Our objectives were to construct a new bacterial detection system using SA-ISFET and to verify its effectiveness using a model system targeting *E. coli*. Additionally, we evaluated its applicability to multiple bacterial species isolated from environmental samples, and examined its potential for multiplex detection using a single low-specificity antibody with broad cross-reactivity.

2. Materials and Methods

This study aimed to develop a rapid and user-friendly microbial detection method as an alternative to the conventional ELISA approach by integrating antigen antibody reactions with an SA-ISFET sensor. To this end, we systematically conducted selected target microorganisms, prepared antibodies, and constructed sensor-based detection system. The procedure was as follows:

Selection and identification of the target bacteria

For fundamental evaluation of the detection system, *E. coli* K-12 (NBRC 3301), a widely used hygiene indicator organism, was selected. In addition, to explore practical applications, samples were collected from 26 surfaces (e.g., cutting boards, handles, and knives) in a wholesale market that handles fresh food. Following cultivation, environmental bacterial strains were isolated using the dilution plate method and incubated at 37°C for 48 h.

The isolated microorganisms were identified by combining matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Bruker Microflex) with BLAST analysis of the 16S rRNA gene sequences. For MALDI-TOF/MS, bacterial samples were mixed with the matrix agent α-cyano-4-hydroxycinnamic (α-HCCA, Bruker Daltonics, USA) and analyzed to obtain mass spectra. The resulting spectra were compared with a microbial library maintained by the NBRC, which contained over 95,000 registered strains. The 10 most frequently identified bacterial species were selected for subsequent experiments (Table 1). All strains were obtained from the Biological Resource Center, National Institute of Technology and Evaluation (NBRC).

Preparation of antibodies

For each of the 10 selected bacterial species (Table 1), heat-inactivated whole cells were used as antigens to immunize individual mice (one strain per mouse) for the production of polyclonal antibodies. After washing the bacterial cells thrice with PBS, they were resuspended in 0.25 mL PBS and mixed with an

Table 1 Environmental bacteria group selected from the high frequently isolated bacteria.

Bacteria ID	Bacteria species
A	*Brochothrix thermosphacta*
B	*Rhodococcus erythropolis*
C	*Kocuria rhizophila*
D	*Microbacterium liquefaciens*
E	*Pseudomonas fluorescens*
F	*Staphylococcus warneri*
G	*Psychrobacter immobilis*
H	*Arthrobacter globiformis*
I	*Pseudarthrobacter oxydans*
J	*Glutamicibacter protophormiae*

equal volume of complete Freund's adjuvant for intraperitoneal injection (BALB/c mice, 7 weeks old, female; Oriental BioService, Kyoto, Japan). Booster immunization was administered at weeks 2, 4, 6, and 8 using incomplete Freund's adjuvant. Serum antibody titers were evaluated by ELISA using bacterial cells immobilized on 96-well plates. Whole blood was collected from mice exhibiting increased titers, and the antibodies were purified using a Protein A affinity column (GE Healthcare). All animal experiments were conducted in accordance with the Guidelines for Animal Welfare and Experimentation at Nara Medical University (approval no. 12215).

Measurement of enzymatic activity using the SA-ISFET

Enzymatic activity was assessed using horseradish peroxidase (HRP), and pH changes were detected using an SA-ISFET sensor (AMIS-051; Bio-X Inc., Kyoto, Japan). Each chip had two independent 50 µL chambers, with one serving as the reaction chamber and the other as the reference. The differential output was quantified (Fig. 2). The reaction buffer contained 1 mmol/L Tris-HCl (pH 7.0), 50 mmol/L NaCl, 0.1% BSA, and 0.02% Tween 20. All measurements were performed at 37°C using an AMIS-101S device (Bio-X Inc.).

SA-ISFET measurements based on antibody-dependent assay

The detection principle was based on the binding

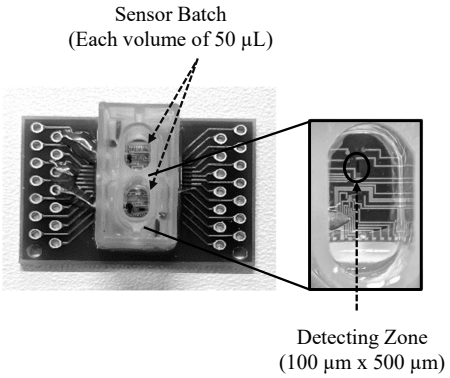

Fig. 2. SA-ISFET sensor structure The SA-ISFET sensor has two reaction chambers of 100 µL capacity on a single sensor chip, each with a 100 µm x 500 µm sensing area in the reaction layer. An enzyme reaction was performed in one of the chambers, and the other chamber was used as a reference chamber where no enzyme reaction occurred, the difference between the sensor outputs of the two chambers was detected as an enzyme reaction.

of HRP-labeled secondary antibodies to primary antibody–bacteria complexes. After filtration, the residual HRP activity in the unbound secondary antibodies served as a detection signal. HRP-conjugated secondary antibody (0.1–2.0 µg/mL) was added to the reaction chamber and preincubated for 1 min. Then, 5 µL of a substrate solution containing 10 mmol/L ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) and 0.1% H_2O_2 was added.

A chamber without reaction was used as a reference. The SA-ISFET output variation was recorded starting from 5 min after the substrate addition, and the average change over 2 min was used for the analysis.

E.coli detection assays

The assay method is illustrated in Fig. 3. To evaluate the detection system, commercially available anti-*E. coli* antibody (ViroStat 1001) and HRP-conjugated anti-rabbit IgG secondary antibody (Abcam, ab97051) were used. After incubating *E. coli* K-12 (NBRC 3301) cell suspension with the primary antibody for 15 min at 20°C, the HRP-labeled secondary antibody (Abcam, ab97051, UK) was added and incubated for an additional 15 min. The reaction mixture was filtered through a 0.45 µm filter, and the filtrate was

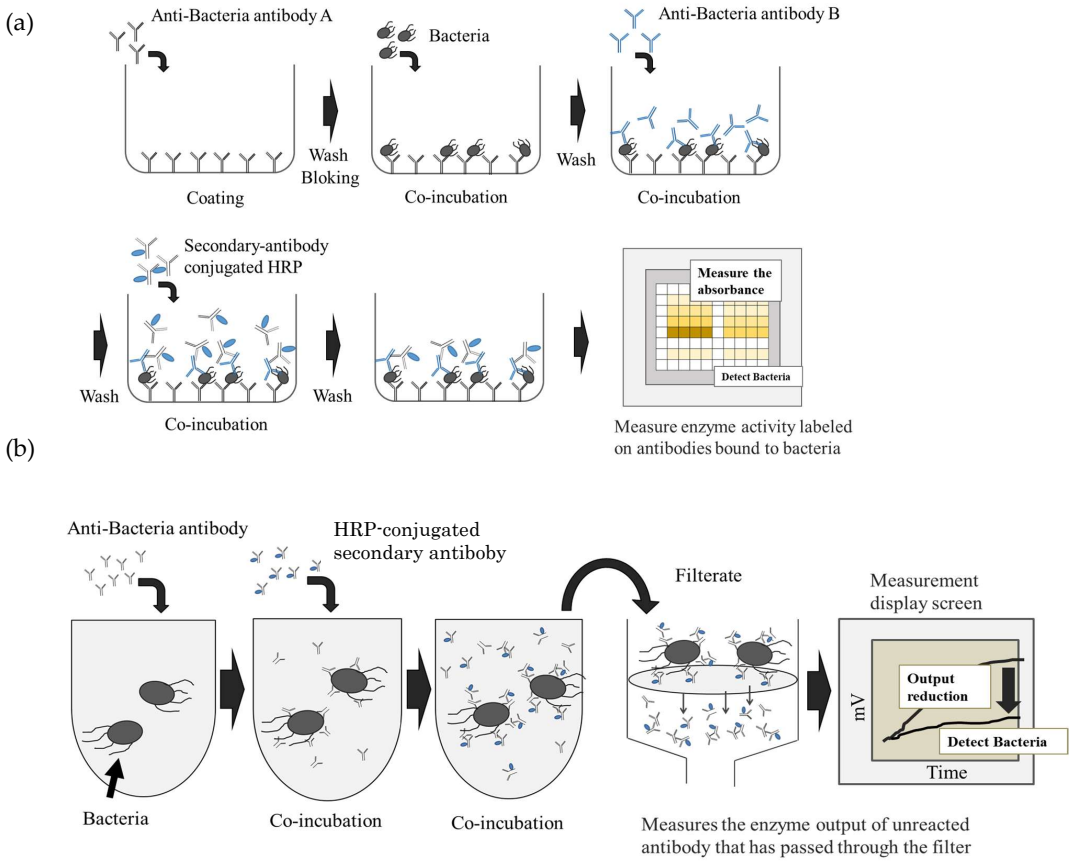

Fig. 3. Comparison of conventional sandwich ELISA with the novel SA-ISFET-based detection method Schematic comparison between the conventional sandwich ELISA method (a) and the newly developed SA-ISFET-based bacterial detection method (b). The SA-ISFET method enables detection based on the remaining enzymatic activity after filtration, whereas ELISA requires immobilization and multiple washing steps.

used for SA-ISFET measurement.

Detection of environmental bacteria

Using the same detection principles and procedures established for *E. coli*, an assay was performed on bacteria isolated from a fresh fish market. Bacterial suspensions were prepared in reaction buffer and mixed with the corresponding primary antibody, followed by 15-min incubation at 20°C to allow antigen–antibody binding. Subsequently, HRP-labeled anti-mouse IgG secondary antibody (Abcam, ab6789) was added and incubated for another 15 min. The solution was filtered (0.45 μm), and HRP activity in the filtrate was measured using the SA-ISFET sensor to indirectly evaluate bacterial presence.

3. Results

Principles and features of the novel detection system

In this study, we devised a novel bacterial detection method based on combining SA-ISFET with immunoassay technique. The detailed procedure of this method was described in the Materials and Methods section and compared to that of a conventional method. The result is summarized in Fig. 3. A conventional method for bacterial detection is sandwich ELISA (Fig. 3a), in which the target bacteria are captured by immobilized antibodies, followed by the binding of enzyme-labeled antibodies to enable quantitative detection based on enzymatic activity. However, this approach requires immobilization and multiple washing steps,

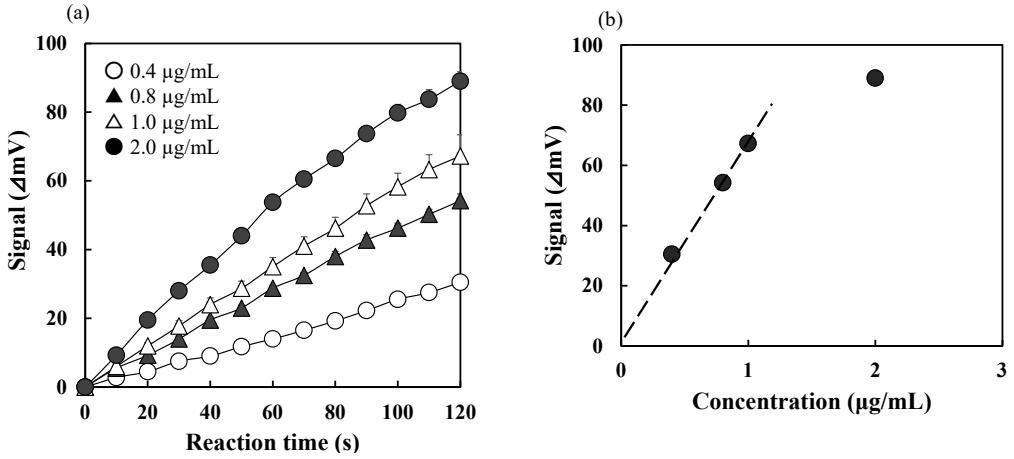

Fig. 4. Time course and analytical curve of horseradish peroxidase (HRP)-conjugated anti-rabbit secondary antibody reaction and its sensor output. The sensor output was measured for 2 min as described in Materials and Methods. (a) Average sensor outputs (in mV) measured over 2 min for different concentrations of anti-rabbit IgG-HRP: 0.4, 0.8, 1.0, and 2.0 μg/mL. Data represent mean ± SD of four sensor outputs. Potential signals were obtained at 0.4 μg/mL (open circles), 0.8 μg/mL (filled triangles), 1.0 μg/mL (open triangles), or 2.0 μg/mL (filled circles). (b) Output plots after 2 minutes of measurement at each concentration based on antibody-labeled HRP activity. Linear relationship between HRP concentration and sensor output observed up to 1.0 μg/mL.

resulting in procedural complexity. To overcome these limitations, we developed a novel detection system in which the presence of bacteria was indirectly evaluated by measuring the decrease in enzymatic activity. In this method, bacteria sequentially reacted with a primary antibody and an HRP-labeled secondary antibody. The resulting mixture was then filtered through a 0.45 μm membrane to remove the bacteria–antibody complexes. The enzymatic activity derived from the unbound HRP-labeled antibodies in the filtrate was subsequently measured using an SA-ISFET sensor (Fig. 3b). Furthermore, the SA-ISFET repeatedly accumulated measurement signals and amplified the output, thereby increasing the S/N ratio and enabling high-sensitivity detection of min changes that could not be detected with the ISFET.

This system enabled rapid and straightforward bacterial detection based on antigen–antibody reactions in the liquid phase, eliminating the need for immobilization or washing steps.

Detection of *E. coli* using anti-*E. coli* antibody

The fundamental performance of the detection system was evaluated using *E. coli* K-12 (NBRC 3301) obtained from NBRC. To assess the dynamic range of the assay, HRP-labeled anti-rabbit IgG secondary antibody was serially diluted from 0.4 to 2.0 μg/mL, and the SA-ISFET sensor output was monitored over a 2-min period (Fig. 4a). The average output of the measurement after 2 min at 0.4 μg/mL was 30.5 ± 5.48 mV (n=4), at 0.8 μg/mL was 54.25 ± 8.69 mV (n=4), at 1.0 μg/mL was 67.25 ± 3.86 mV (n=4), and at 2.0 μg/mL was 89.00 ± 3.11 mV (n=4), demonstrating a linear response up to 1.0 μg/mL (Fig. 4b). Based on these results, a concentration of 0.4 μg/mL was used for subsequent bacterial detection. Next, the constructed detection system was applied to *E. coli* K-12 cells. In the absence of bacteria (control), the average sensor output of the measurement after 2 min based on HRP activity was 20.0 ± 4.85 mV (n=5). In contrast, samples containing 5.0×10^5 cfu/mL E. coli showed a decreased output of 8.80 ± 6.42 mV (n=5), whereas those with 2.5×10^6 cfu/mL showed negligible output (Fig. 5a). To further evaluate the correlation between *E.coli* concentration and SA-ISFET output, the response (mV) was plotted on the y-axis against bacterial concentration on the x-axis using a logarithmic scale (Fig. 5b). Because zero values cannot be represented on a logarithmic axis, the zero-concentration control was displayed separately in an inset with a linear scale. The result showed a decrease in SA-ISFET output based on the enzyme reaction with bound antibody, consistent with the measurement principle that higher bacterial counts reduce the amount of antibody passing

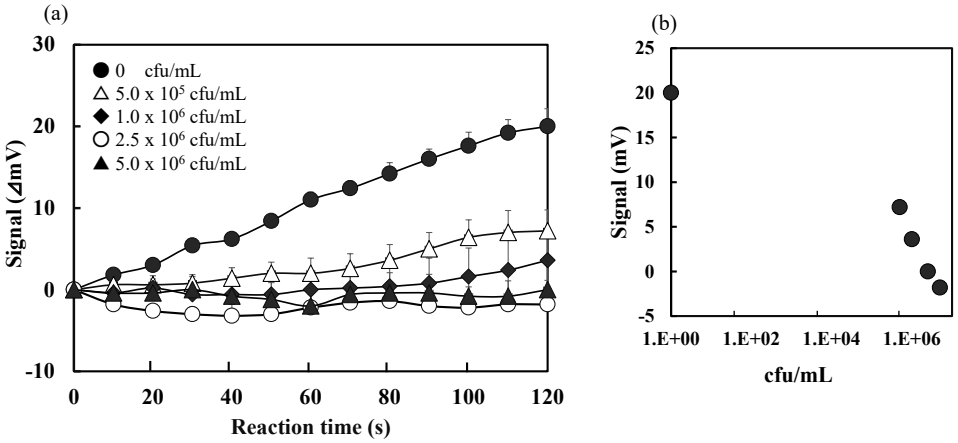

Fig. 5. SA-ISFET sensor output based on the enzymatic activity of unbound HRP-labeled secondary antibodies after filtration, in response to various *Escherichia coli* concentrations. (a) Detection of *E. coli* by immunoreactivity and SA-ISFET. Time course of reactions and changes in signal accumulation. The sensor output was measured for 2 min as described in Materials and Methods. The HRP-conjugated anti-*E. coli* secondary antibody concentration of 0.4 μg/mL. Data shown include control (filled circles), 5.0×10^5 cfu/mL (open triangles), 1.0×10^6 cfu/mL (filled diamonds), 2.5×10^6 cfu/mL (filled triangles), and 5.0×10^6 cfu/mL (open circles). Data represent mean ± SD of four sensor outputs. (b) The response was proportional to bacterial number; however, strict linearity was not observed because antibody binding and filter capture limited the amount of unbound enzyme-labeled antibodies available for detection. For plotting on the logarithmic axis, zero values were substituted with 1 cfu.

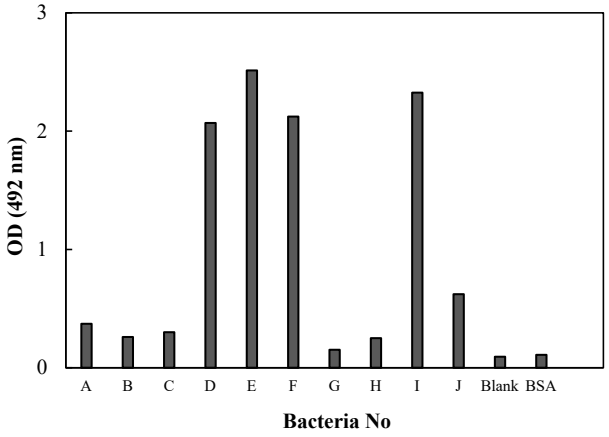

Fig. 6. Evaluation of the cross-reactivity between killed cells and antibody (J8) in the culture supernatant. The killed bacteria A-I (Table 1) were immobilized in 96-well plates and evaluated for cross-reactivity with the J8 antibody.

through the filter. However, strict linearity was not observed across the entire concentration range.

Selection of bacterial strains and screening of detecting antibodies

To develop an assay for detecting environmental bacteria, samples were collected from 26 hygienic sites such as knives, cutting boards, and handles. Following cultivation on agar plates, isolates were identified using 16S rRNA gene sequencing and MALDI-TOF MS. The 10 most frequently identified species were selected as targets for this study (Table 1), and polyclonal antibodies were prepared for each. Cross-reactivity tests revealed that the J8 antibody, raised against *Glutamicibacter protophormiae*, exhibited significant reactivity with all 10 target species (Fig. 6). Strong reactivity was observed

against *Microbacterium liquefaciens* (D), *Pseudomonas fluorescens* (E), *Staphylococcus warneri* (F), and *Pseudarthrobacter oxydans* (I). Therefore, the J8 antibody was selected for further experiments.

Detection of environmental bacteria using J8 antibody

To verify the linearity of the detection system using J8 and HRP-labeled anti-mouse IgG, the antibody was serially diluted from 0.1 to 0.4 μg/mL. 2-min sensor output measurements yielded 25.2 ± 5.9 mV (n=4) at 0.1 μg/mL, 52.8 ± 3.87 mV (n=4) at 0.2 μg/mL, and 82.3 ± 5.74 mV (n=4) at 0.4 μg/mL, demonstrating linearity up to 0.2 μg/mL (Fig. 7a, b). Accordingly, 0.1 μg/mL was used in subsequent detection assays. Detection assays were conducted for four bacterial species showing strong cross-reactivity with J8 at varying concentrations to examine their impact on sensor output. For *M. liquefaciens* (D), the control output was 23.75 ± 2.22 mV (n=4). In contrast, samples with 1.5×10^5 cfu/mL, 1.5×10^6 cfu/mL, and 3.0×10^6 cfu/mL showed decreasing outputs of 17.00 ± 3.37 mV, 13.75 ± 2.50 mV, and 8.25 ± 5.06 mV, respectively (Fig. 8a). Similarly, for *P. fluorescens* (E), the control value was 23.75 ± 2.22 mV, and values of 19.00 ± 0.82 mV, 16.80 ± 1.79 mV, and 11.00 ± 3.27 mV were recorded at 1.5×10^5, 1.5×10^6, and 3.0×10^6 cfu/mL, respectively (Fig. 9a). For *S. warneri* (F), the control was 23.75 ± 2.22 mV. Output decreased to 12.40 ± 2.79 mV at 1.5×10^5 cfu/mL, 9.25 ± 4.19 mV at 7.5×10^5 cfu/mL, and 3.20 ± 4.49 mV at 1.5×10^6 cfu/mL (Fig. 10a). For *P. oxydans* (I), the control output was 30.20 ± 7.83 mV. In samples with 1.1×10^5, 1.1×10^6, and 2.7×10^6 cfu/mL, the outputs were 24.75 ± 1.71 mV, 5.75 ± 2.08 mV, and 4.50 ± 4.57 mV, respectively, indicating substantial signal attenuation (Fig. 11a). In addition, the SA-ISFET output after 2 minutes of measurement was plotted against the number of bacteria, as in the measurement with *E. coli*. The plots of SA-ISFET output based on the number of bacteria were shown in Fig. 8b for *M. liquefaciens*, Fig. 9b for *P. fluorescens*, Fig. 10b for *S. warneri*, and Fig. 11b for *P. oxydans*.

These results clearly demonstrate that, although the SA-ISFET output did not show strict concentration linearity for all tested species, it decreased in a concentration-dependent manner. Although specificity was low, the J8 antibody enabled broad-range detection of multiple bacteria using a single assay configuration.

4. Discussion

In this study, a novel bacterial detection method was developed by integrating a SA-ISFET with antigen–antibody reactions, and its effectiveness was

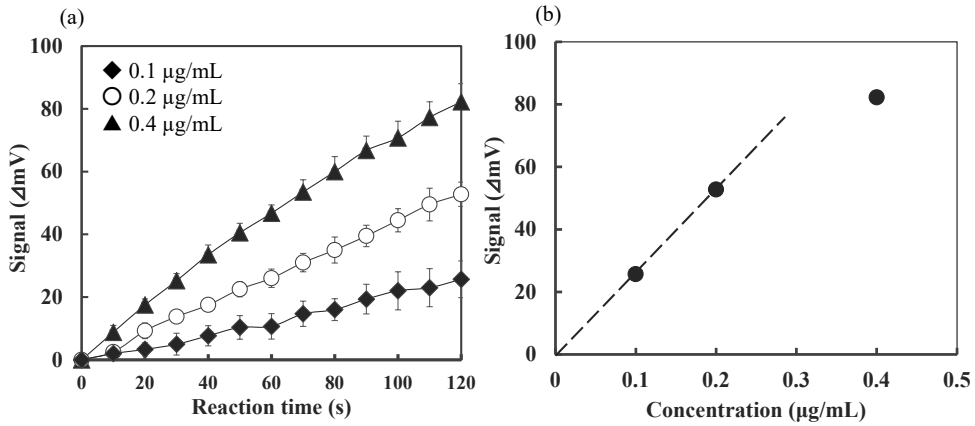

Fig. 7. Time course and analytical curve of horseradish peroxidase (HRP)-conjugated anti-mouse secondary antibody reaction and sensor output. The sensor output was measured for 2 min, as described in Materials and Methods. (a) Potential signals were obtained at 0.1 μg/mL (filled diamonds), 0.2 μg/mL (open circles), or 0.4 μg/mL (filled triangles). (b) Output plots after 2 minutes of measurement at each concentration based on antibody-labeled HRP activity. Linear relationship between HRP concentration and sensor output observed up to 0.2 μg/mL.

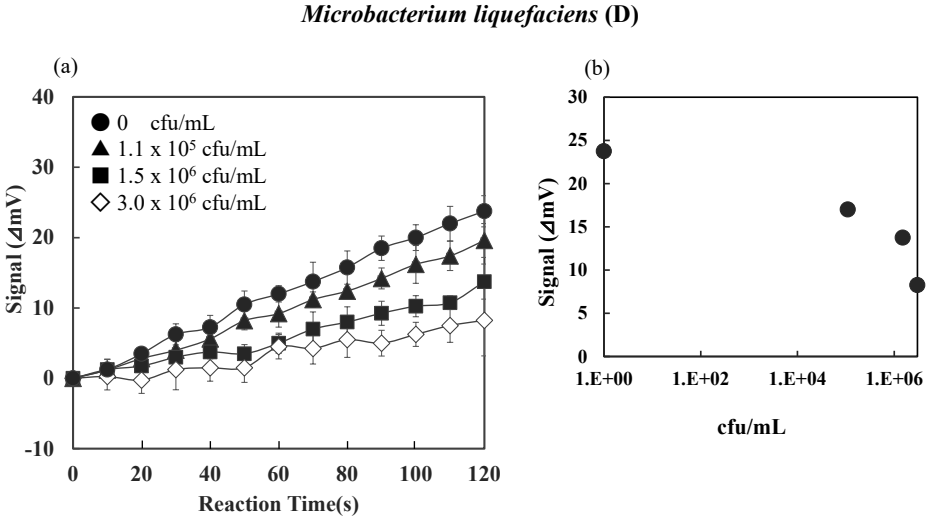

Fig. 8. SA-ISFET sensor output based on the enzymatic activity of unbound HRP-labeled secondary antibodies after filtration, in response to *M. liquefaciens* (D) concentrations. (a) The sensor output was measured for 2 min as same as for *E. coli* measurement. The HRP-conjugated anti-J8 secondary antibody concentration of 0.1 μg/mL. Data shown include control (filled circles), 1.1 × 10^5 cfu/mL (filled triangles), 1.5 × 10^6 cfu/mL (filled squares), and 3.0 × 10^6 cfu/mL (open diamonds). Data represent mean ± SD of four sensor outputs. (b) Output plots after 2 minutes of measurement based on free antibody-labeled HRP activity passing through the filter at each bacterial concentration. For plotting on the logarithmic axis, zero values were substituted with 1 cfu.

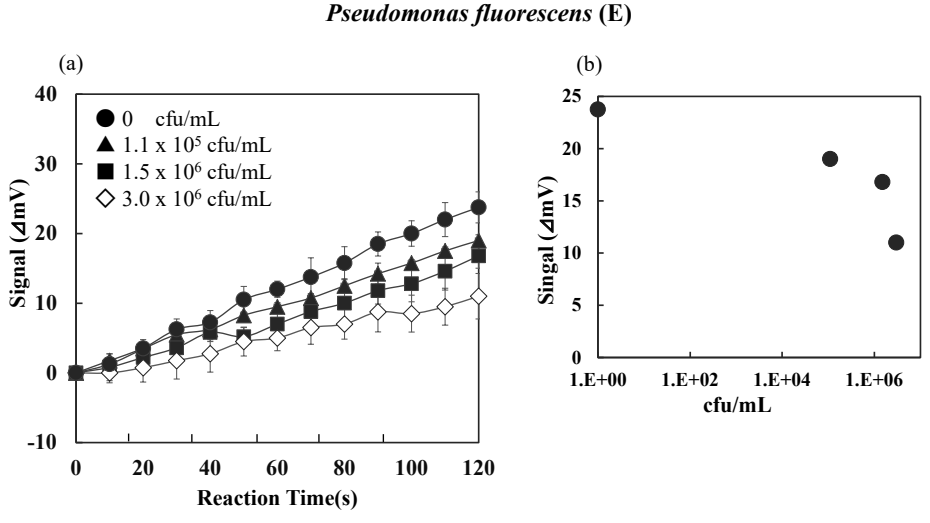

Fig. 9. SA-ISFET sensor output based on the enzymatic activity of unbound HRP-labeled secondary antibodies after filtration, in response to various *P. fluorescens* (E) concentrations. (a) Sensor output over 2 min under the same conditions as in Fig. 8. Data shown include control (filled circles), 1.1 × 10^5 cfu/mL (filled triangles), 1.5 × 10^6 cfu/mL (filled squares), and 3.0 × 10^6 cfu/mL (open diamonds). Data represent mean ± SD of four sensor outputs. (b) Output plots after 2 min of measurement based on free antibody-labeled HRP activity passing through the filter at each bacterial concentration. For plotting on the logarithmic axis, zero values were substituted with 1 cfu.

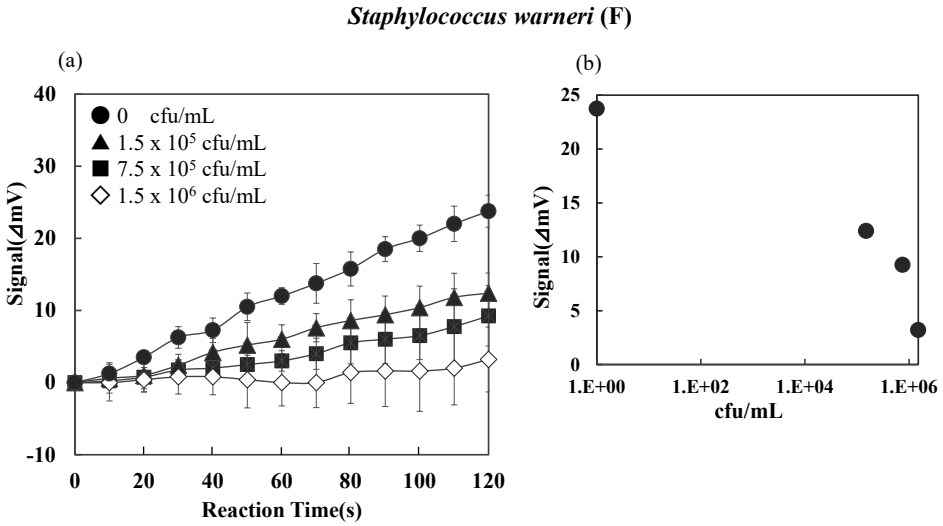

Fig. 10. SA-ISFET sensor output based on the enzymatic activity of unbound HRP-labeled secondary antibodies after filtration, in response to various *S. warneri* (F) concentrations. (a) Sensor output over 2 min under the same conditions as in Fig. 8. Data shown include control (filled circles), 1.5 × 10^5 cfu/mL (filled triangles), 7.5 × 10^5 cfu/mL (filled squares), and 1.5 × 10^6 cfu/mL (open diamonds). Data represent mean ± SD of four sensor outputs. (b) Output plots after 2 min of measurement based on free antibody-labeled HRP activity passing through the filter at each bacterial concentration. For plotting on the logarithmic axis, zero values were substituted with 1 cfu.

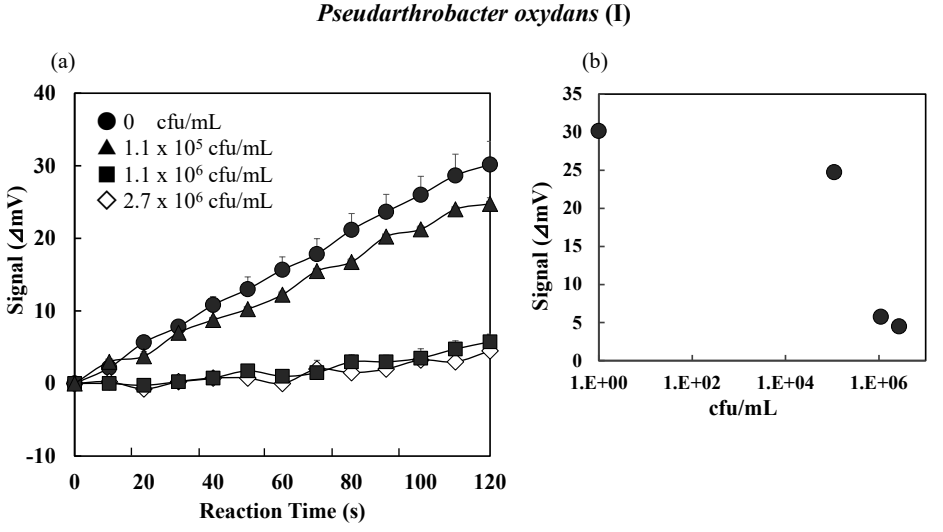

Fig. 11. SA-ISFET sensor output based on the enzymatic activity of unbound HRP-labeled secondary antibodies after filtration, in response to various *P. oxydans* (I) concentrations. (a) Sensor output over 2 min under the same conditions as in Fig. 8. Data shown include control (filled circles), 1.1 × 10^5 cfu/mL (filled triangles), 1.1 × 10^6 cfu/mL (filled squares), and 2.7 × 10^6 cfu/mL (open diamonds). Data represent mean ± SD of four sensor outputs. (b) Output plots after 2 min of measurement based on free antibody-labeled HRP activity passing through the filter at each bacterial concentration. For plotting on the logarithmic axis, zero values were substituted with 1 cfu.

successfully demonstrated. Unlike conventional detection methods such as sandwich ELISA, which require immobilization of antibodies and multiple washing steps, the proposed method combines physical filtration-based separation with electrical detection of pH changes using SA-ISFET[13], enabling a simplified and highly sensitive detection system. One of the most notable advantages of this system is that one of the features of the SA-ISFET sensor is that it is label-free[8], which greatly simplifies the assay by eliminating the need for antibody immobilization. Second, the use of only two-step reactions involving a primary antibody and an HRP-labeled secondary antibody reduces the burden of antibody selection and preparation, offering cost-effectiveness and time- efficiency. Third, the elimination of washing steps and the use of direct filtration of the reaction mixture enabled the reproducible separation of unbound antibodies. Furthermore, the high signal-to-noise (S/N) ratio and signal accumulation capability of the SA-ISFET sensor allowed the stable detection of subtle pH changes resulting from enzymatic reactions, underscoring the technical advantage of this method.

A major achievement of this study was the strategic utilization of low-specificity antibody with broad cross-reactivity, which allowed the detection of multiple bacterial species using a single antibody. Although there have been some studies on detection methods for multiple species of bacteria[14], high specificity is usually desirable for antibody-based detection. However, in this study, cross-reactive antibodies (J8) were purposely employed to develop a widely applicable screening system. Using this approach, successful detection of several species, including *M. liquefaciens*, *P. fluorescens*, *S. warneri*, and *P. oxydans*, was demonstrated, indicating the feasibility of multiplex bacterial detection.

A limitation of this assay at this stage, however, was that while the ISFET output decreased in proportion to the number of bacteria, it did not show strict linearity over the entire range. This nonlinearity is inherent to the assay principle. The principle states that as the bacterial concentration increases, more antibodies are captured with the bacteria and retained on the filter, thereby reducing the number of unbound enzyme-labeled antibodies passing through. Because the amount of antibody present in the reaction solution is limited, once a certain number of bacteria is exceeded, most of the antibody is already bound, leaving little unbound antibody. In this region, the output may not continue to decrease in proportion to the number of bacteria, but rather may show a rapid decay. As a result, the quantitative dynamic range of the method was limited and might be considered non-linear.

In addition, the concentration and signal intensity of the J8 antibody vary among the bacteria, and the zero-concentration sample values also vary among species. Therefore, it is always advisable to measure a blank and take the difference. Since this study is still preliminary, further optimization is needed for practical application. This includes reducing these effects and achieving more stable sensor outputs through improvements in reagents, software signal processing, and device design. To enhance the sensitivity, improvements in antibody affinity, antibody recovery efficiency after filtration, and differential analysis of sensor outputs are required. Moreover, to reduce batch-to-batch variability in antibody performance[15], the introduction of monoclonal or recombinant antibody technologies may be promising. Although the current SA-ISFET measurement system relies on benchtop instrumentation, future integration with microfluidic channels and miniaturization may enable portable, on-site detection. During the LAMP reaction, protons (H^+) are released during DNA synthesis, causing the pH of the reaction solution to decrease. This pH change is directly reflected on the sensing surface of the SA-ISFET and recorded as a potential change, enabling label-free tracking of the amplification reaction's progress. Therefore, using SA-ISFET enables direct, real-time detection of nucleic acid amplification without relying on indirect methods such as fluorescent dyes or turbidity measurements. Consequently, the incorporation of nucleic acid amplification methods, such as LAMP, and the implementation of real-time signal processing algorithms may further enhance sensitivity and broaden applicability to infection screening and food safety testing[16,17].

A model test using environmental bacteria

selected in a food sanitation environment showed that this method can detect bacteria at 5.0 x 10^5cfu/mL. This level aligns with the microbial limit for general foods (e.g., boxed meals, raw foods) specified in the Japanese food sanitation standards (MHW Notification No. 370, 1959), which sets the acceptable aerobic plate count at 10^5 cfu/g[18]. This suggests the applicability of the method for hygiene assessment and cleanliness monitoring in food production environments. Furthermore, the global COVID-19 pandemic has increased the awareness of hygiene management and infectious disease prevention. The WHO guidelines emphasize strengthened monitoring of water quality, sanitation, and the environment, and microbial detection technologies for environmental and foodborne pathogens are now central to public health strategies[19]. The detection method developed in this study aims to be applied to the detection of viruses, pesticides, and other environmental contaminants. However, it is currently at a preliminary stage, and several issues remain to be addressed, such as eliminating the effects of factors like sample pH and buffering capacity on sensing when used with real samples. Further research and development is necessary to address these challenges.

In summary, the SA-ISFET-based bacterial detection platform developed in this study overcomes the complexities of conventional methods by combining sensor technology with immunochemical recognition. This demonstrates its high practical utility and expandability. Future efforts should focus on developing target-specific antibodies and receptors, optimizing surface modifications of the sensor, and conducting application studies using clinical, food, and environmental samples to accelerate its real-world deployment.

Conflicts of interest

The authors declare no conflicts of interest. The sponsors played no role in the design and, execution of the study, interpretation of the results, or writing of this report.

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