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A compact microfluidic chip with integrated impedance biosensor for protein preconcentration and detection

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In this study, a low-cost, compact biochip is designed and fabricated for protein detection. Nanofractures formed by self-assembled gold nanoparticles at junction gaps are applied for ion enrichment and depletion to create a trapping zone when electroosmotic flow occurs in microchannels. An impedance measurement module is implemented based on the lock-in amplifier technique to measure the impedance change during antibody growth on the gold electrodes which is caused by trapped proteins in the detection region. The impedance measurement results confirm the presence of trapped proteins. Distinguishable impedance profiles, measured at frequencies in the range of 10–100 kHz, for the detection area taken before and after the presence of proteins validate the performance of the proposed system. Published by AIP Publishing. <https://doi.org/10.1063/1.4996118>

I. INTRODUCTION

Immunoassays are widely applied in medicine and biomedicine. The efficient detection of a low concentration of proteins is important in various applications.^{1,2} Protein quantification is often necessary before the isolation, separation, and analysis of protein samples by chromatographic, electrophoretic, and immunochemical techniques.³ In order to get a sufficient concentration of proteins for detection, proteins can be preconcentrated using electrophoresis.^{4,5} In this technique, proteins can be concentrated in a detection region by applying an electrical voltage to a microfluidic channel. Electrical concentration methods have many advantages such as ease of system fabrication and operation.

The ion exclusion-enrichment effect (EEE) has been applied for protein concentration in immunoassay systems to enhance the detection speed and sensitivity.^{6,7} Ion-selective membranes with the advantage of low applied DC preconcentration voltage were also applied in EEE.^{8–11} The EEE in combination with the surface acoustic wave (SAW) was utilized in cancer diagnosis, which showed its potential for cancer studies.¹² So far, several approaches for creating nanochannels/nanopores to produce the EEE have been reported,¹³ such as photolithography,^{14,15} the integration of commercially available membranes with nanopores,^{4,7} and the employment of junction gap electric breakdown between two polydimethylsiloxane (PDMS) microchannels.^{16–18} In most of these studies, protein concentrations are quantified by using complex and costly fluorescence spectroscopy systems.¹⁹

The impedance measurement, a low-cost method that is easy to integrate, has been applied for protein detection.^{12,20,21} A popular method is electrochemical impedance spectroscopy (EIS).^{22,23} Typically, biochips are measured using expensive EIS systems. The EEE and

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impedance measurement technique have been studied to develop an actuator-detector integrated system with advantages in the simple fabrication process and compact measurement setup.¹²

In our previous work, protein preconcentration was implemented in a biochip based on the exclusion enrichment method and nanostructures were created by breaking down the junction gap between two microfluidic channels activated by gold nanoparticles.²⁴ Recently, a simple protein preconcentrator was proposed,²⁵ in which the EEE is achieved in nanointerstices via a self-assembled monolayer (SAM) of Au nanoparticles. This simple and reliable method does not require a high voltage or time-consuming fabrication.

In the present work, a low-cost design for protein detection was implemented based on a compact biochip with built-in protein preconcentration and impedance measurement functionalities. Proteins in the microfluidic channel are preconcentrated in the sensing area by using the EEE and electroosmotic force (EOF) by applying low DC voltages to the microfluidic channel inlets. The target proteins are trapped onto the electrode surface by the surface-immobilized antibody, while the non-target objects are washed away. The impedance between the two sensing electrodes is changed when target proteins are preconcentrated and trapped in the sensing area, and therefore, the presence of proteins can be detected.

II. MICROFLUIDIC PLATFORM DESIGN FOR PROTEIN PRECONCENTRATION AND DETECTION

Figure 1 shows the structure of the biochip that consists of two functioning components, i.e., actuator and sensor. Four driving electrodes supply a DC voltage to the microfluidic channels through four inlets to form nanostructures for protein preconcentration. Two sensing electrodes are located at the appropriate positions inside the channels to detect the presence of trapped proteins. The actuator design is based on the EEE, the depletion effect, and EOF. Rabbit anti-BSA antibodies are immobilized on a gold electrode for rabbit BSA protein trapping. This proposed microfluidic chip is made of a glass substrate and PDMS microfluidic channels. The layout and fabrication of the proposed protein preconcentrator herein are basically the same as those in our previous work.^{24,25} After PDMS is bonded to glass to form microchannels, surface modification is implemented to immobilize antibodies on the sensing electrodes. The details of the structure are presented in Fig. 1(b). The microfluidic channel has a width of 100 μm , a

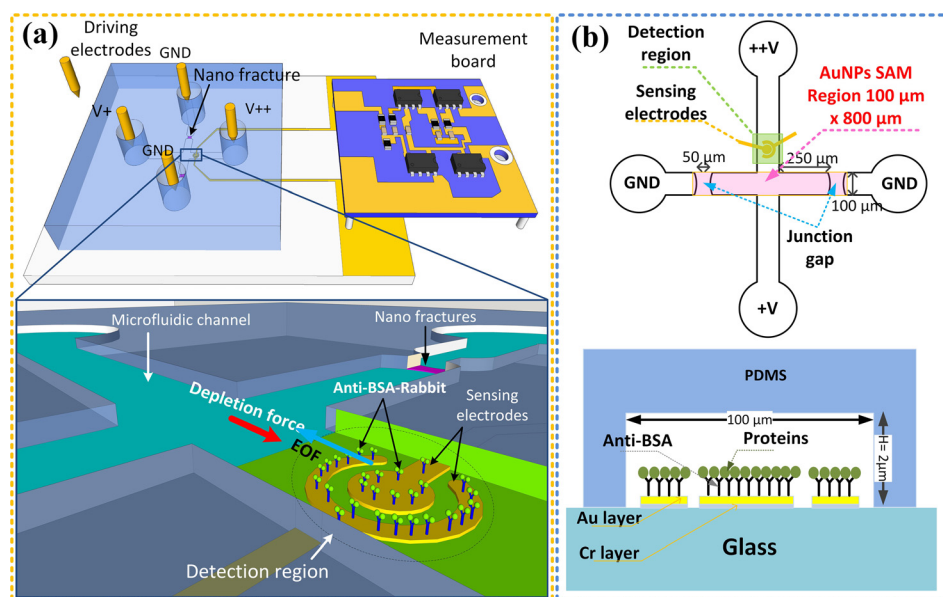


FIG. 1. Schematic of the proposed EEE-based protein concentration with integrated impedance sensing electrodes for protein detection. (a) Highly sensitive lock-in amplifier technique is employed for quantitatively recognizing proteins at the designated detection window. (b) Details of microfluidic channels and gold nanoparticle region.

height of $2\ \mu\text{m}$, and a junction gap of $50\ \mu\text{m}$. The height of the channel is designed to be $2\ \mu\text{m}$ in order to reduce the static incubation time. Furthermore, with this height, we can reduce the volume of the protein sample used in experiments. Nanofissures with dimensions of $800\ \mu\text{m} \times 200\ \mu\text{m}$ were assembled on the glass at the two junction gaps structured by bonding PDMS to glass.

A. Protein preconcentration

By applying appropriate DC voltages at the four inlets, EOF and a depletion force are generated and utilized to preconcentrate proteins to the designated position for detection. After protein preconcentration, the presence of proteins can be detected via the impedance measurement of the two gold electrodes.

In this design, the EEE, the depletion effect, and electroosmotic flow are utilized for protein preconcentration.²⁵ Four inlets and three microchannels are structured with two junction gaps formed by microchannels. Gold nanoparticles are deposited on glass at the junction gaps. Nanofractures are created by applying a high DC voltage (i.e., 50 VDC) between the microchannels through the junction gap. The nanofractures create ion-enrichment and ion-depletion effects when an appropriate DC voltage is applied to the microchannels, which are filled with phosphate-buffered saline (PBS) solution. With an applied voltage, proteins in the microchannels are driven into the detection region, where antibodies are grown on the electrodes, for detection.

Figure 2 briefly describes the protein preconcentration process. After proteins and the PBS buffer solution are injected into the microchannels, a DC voltage is applied to the channels, as shown in Fig. 2(a). The same DC voltage is applied to the two inlets, and the other channels are connected to GND. The DC voltage creates a depletion area due to nanofractures at the junction gaps [Fig. 2(b)] where there are no ions. Ions with a negative charge (including

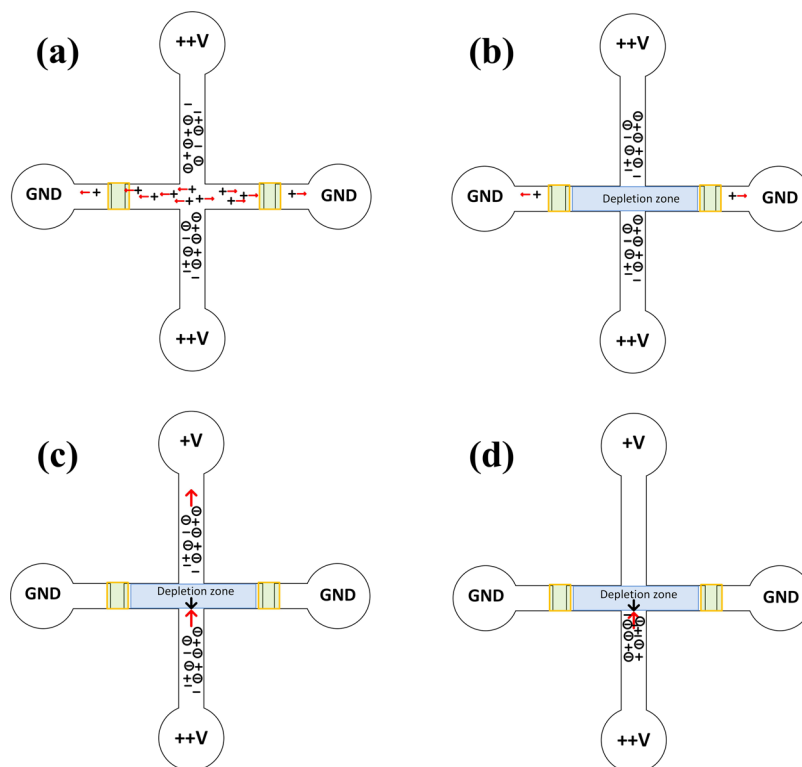


FIG. 2. Process of protein preconcentration based on EEE and electroosmotic flow.²⁵ (a) Nanofractures generated by applying 50 VDC to two inlets and 0 V to other inlets. (b) Nanodepletion zone formation. (c) Electroosmotic flow activation by bias voltage +V. (d) Protein concentration by the depletion zone and electroosmotic flow after bias voltage application.

proteins) are pulled out of the depletion area. A bias voltage is then applied, where a positive voltage (+V) is applied to one side and a more positive voltage (++V) is applied to the other side, as shown in Fig. 2(c). An electroosmotic flow is thus created to move proteins toward the +V inlet [Fig. 2(d)]. By balancing the depletion force and electroosmotic flow, proteins are trapped near the depletion area in the microchannel. Proteins are thus preconcentrated at a detection window for detection by the impedance measurement module.

B. Immunoassay based on FITC-BSA rabbit antibody

In our preview work, protein detection was implemented based on an immunoassay that utilized antibodies and fluorescence spectroscopy.²⁴ In this approach, proteins trapped by antibodies are detected using the impedance method, where the impedance between electrodes is changed due to the presence of the targeted protein. The impedance method is adopted in this study due to its low cost and ease of integration with microfluidic channel structures. The FITC-BSA rabbit antibody used to select proteins is immobilized at the two sensing electrodes. The proteins after preconcentration can thus be selected for immunoassay.

The surface modification process for antibody immobilization was as follows: O-(2-carboxyethyl)-O'-(2-mercaptoethyl) heptaethylene glycol was injected into the microchannels and incubated for 12–18 h. The thiol groups form Au-S bonds on the surface of the sensing electrodes and produce a SAM after exposure to carboxyl groups. The microfluidic channels were then washed with PBS (1 mM). A mixture of 10 mg/ml EDC N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride and 5 mg/ml N-hydroxysuccinimide (dissolved in 1 mM PBS) was injected and incubated for 30 min. This mixture acts as a coupling agent to activate the carboxyl group of the thiolated polyethylene glycol into a reactive ester and form an amide bond with the amino group of anti-BSA on the SAM. The microchannels were washed after the activation of the functional groups by injecting 0.2 mg/ml anti-BSA into them. Anti-BSA was incubated for 30 min to allow the formation of an amide bond with the SAM. The method is efficient when the concentration of proteins is high (i.e., an optical method can be used to observe the presence of proteins captured by antibodies). For low protein concentrations, protein preconcentration should be implemented to enhance the concentration of proteins in a designated measurement region. Proteins can then be enriched and easily detected using the impedance measurement.

C. Biosensing based on the impedance measurement

Impedance detection works based on the change in impedance between electrodes when proteins are captured by antibodies on the gold electrodes. A low-cost, compact design based on an analog lock-in amplifier is applied in the impedance measurement. Figure 3(a) presents a model of the impedance measurement method, where the microfluidic channel is fully filled with PBS solution to increase the conductivity between the electrodes. The impedance of the biochip is defined by three components, namely, R_s , R_f , and C_f . Assume that the electrodes and PBS solution medium form a double-layer capacitance (C_f) and resistance R_f of the surface impedance. The PBS solution can be represented as a resistance (R_s) connected in series with the surface impedance.

Suppose that proteins captured by antibodies change the total impedance due to the change in conductivity and capacitance (R_f and C_f) [Fig. 3(b)]. The total impedance of the biosensor can be calculated as

$$Z = R_s + \frac{R_f}{\omega^2 \tau^2 + 1} - \frac{\omega R_f^2 C_f}{\omega^2 \tau^2 + 1} j, \quad (1)$$

where $\tau = R_f C_f$. The series capacitance C_s can be extracted from the imaginary component of the impedance in Eq. (1), which is given by

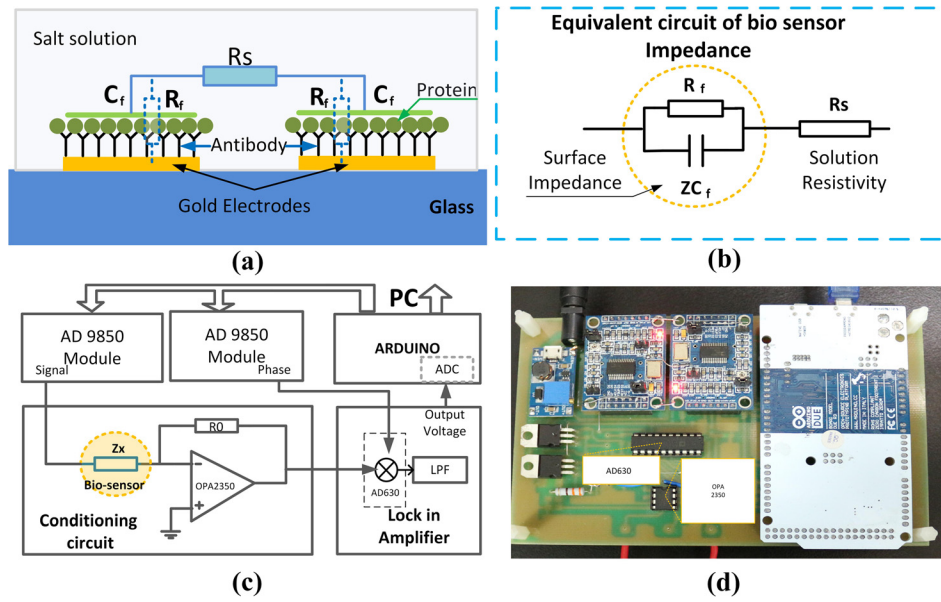


FIG. 3. Impedance biosensor based on the impedance measurement method. (a) Model of the impedance biosensor based on the impedance measurement method. The impedance includes the surface impedance and resistance (from PBS). Gold electrodes and PBS form surface impedance, which consists of capacitance C_f and resistance R_f . R_s is the resistivity of PBS solution. (b) Equivalent circuit of the biosensor consisting of surface impedance and solution resistance. (c) Block diagram of the impedance measurement module. A microcontroller is used to adjust the frequency and phase through two AD 9805 function generator ICs and for data acquisition. The impedance measurement is implemented using IC OPA2350 for the balance-bridge conditioning circuit. AD 630 is used for analog lock-in amplification. (d) An actual image of the printed circuit board of the impedance measurement module.

$$C_s = C_f + \frac{1}{\omega^2 C_f R_f^2}. \quad (2)$$

As can be seen, C_s is a function of frequency. At high frequencies, C_s is approximately C_f . However, at low frequencies, C_s depends on ω , C_f , and R_f . In this proposed impedance sensor structure, the C_f and R_f parameters are changed when the target proteins are trapped on the electrode surface. Both the real and imaginary components in Eq. (1) are consequently changed. Therefore, the presence of target trapped proteins can be detected and concentration can be estimated by monitoring the change in real and imaginary parts of impedance profiles. Figure 3(c) shows the block diagram of the proposed impedance measurement module. An Arduino board is used to control and acquire measurement data. Two AD9805 modules are used with a synchronized clock to generate a signal with 0° and 90° phase shifts and frequencies in the range of 10–100 kHz. An OPA2350 IC is used for the pre-amplifier as a conditioning circuit, and an AD630 IC is used for phase detection in the lock-in amplifier block. Two 12-bit analog-to-digital converters (ADCs) in the Arduino DUE 2013 R3 convert the output signals for data acquisition. The output voltages of 0° and 90° phases of the lock-in amplifier are used to calculate the range of impedance and the resistivity and capacitance of the biosensor.

III. EXPERIMENTAL EVALUATION

First, the preconcentration performance of the biochip was confirmed using the fluorescence technique.²⁵ The microfluidic channel was washed and fully filled with 1 mM PBS buffer solution. After all the channels were cleaned, $2 \mu\text{l}$ of fluorescein isothiocyanate conjugated to bovine serum albumin (FITC BSA; $10 \mu\text{M}$) was injected into the ++V inlet and $58 \mu\text{l}$ of PBS solution was injected into the +V inlet. Then, $20 \mu\text{l}$ of PBS solution was injected into the GND inlets, as shown in Fig. 1(a). The protein preconcentration is implemented by applying a driving voltage to the microfluidic channels at four inlets using a power supply (Series 225, Bertan High

Voltage Corp., Hicksville, NY, USA). Two GND inlets are connected to 0 V, the ++V inlet is connected to 50 VDC, and the +V inlet is connected to 48 VDC [Fig. 1(a)].

After evaluating the preconcentration performance of the chip, the impedance of the biosensor was characterized to determine trapped proteins in the detection region. The impedance measurement is also used to investigate the binding of rabbit BSA antibodies on the gold electrodes. Measurements were taken before and after the surface modification process for antibody immobilization to confirm the antibody binding on the gold electrodes.

The impedance measurement module records the impedance data of the sensing element before and after the immobilization of rabbit anti-BSA antibodies and after the proteins were selectively captured on gold electrodes. The impedance was measured with time for evaluating the appropriate waiting time needed for antibody growing and protein capturing processes. The measurement results are reported in Sec. IV.

IV. EXPERIMENTAL RESULTS AND DISCUSSION

A. Fluorescence result to confirm the presence of target proteins

The preconcentration effect was confirmed experimentally. Fluorescence images of the 10 μ M FITC-BSA rabbit in a 10 mM PBS solution taken at various time points are shown in Fig. 4. The concentration of BSA increased with time, confirming the electrokinetic protein preconcentration. The protein is trapped in the detection region after supplying 0 V, 50 DC voltage, and 48 DC voltage based on depletion force, and EOF is shown in Fig. 4(a). Figure 4(b) shows the protein preconcentration obtained during the low DC voltages supplied to the microfluidic channels after 10, 20, and 30 min. As can be seen, fluorescence densities after 20 and 30 min are the same, confirming that 20 min is enough for proteins concentrated to the detection region. The initial concentration of BSA is 10 μ M. After 20 min applying of preconcentration voltage, the concentration is confirmed to be more than 0.5 mM (50 Fold).²⁵

B. Impedance measurement result to confirm the presence of the antibody immobilized on sensing electrodes

Figure 5(a) shows the impedance profile of the fabricated devices after antibody immobilization on the gold electrodes. An exciting signal with an amplitude of 600 mV_{p-p} is applied in this measurement. The results reveal that the impedance of the sensing element has a small deviation, i.e., the relative standard deviation is less than 9.5% between chips under the same measurement conditions. At a measurement frequency of above 30 kHz, the deviation is even smaller, i.e., less than 4.0%. Figure 5(b) shows the results for the fourth sample taken before

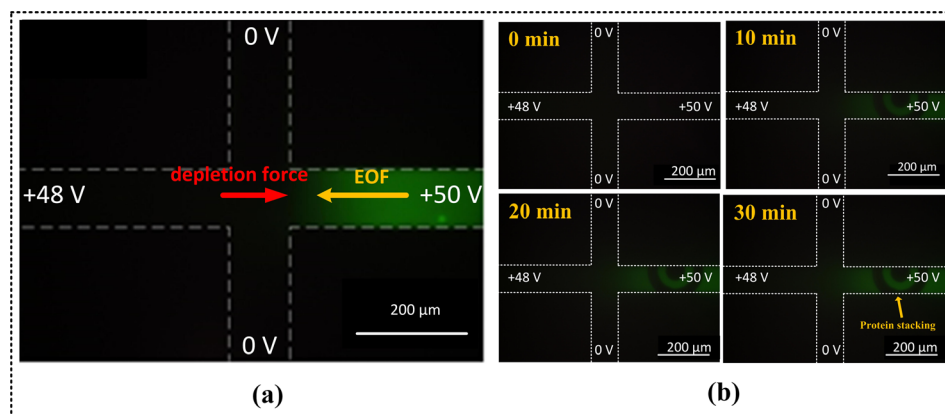


FIG. 4. Protein distribution when DC voltages are applied. Proteins are concentrated at the designated detection window after 30 min. (a) The protein is trapped in the detection region after supplying 0 V, 50 DC voltage, and 48 DC voltage. The trap works based on depletion force and EOF. (b) Successful protein preconcentration obtained after 30 min low DC voltages were supplied to the microfluidic channels.

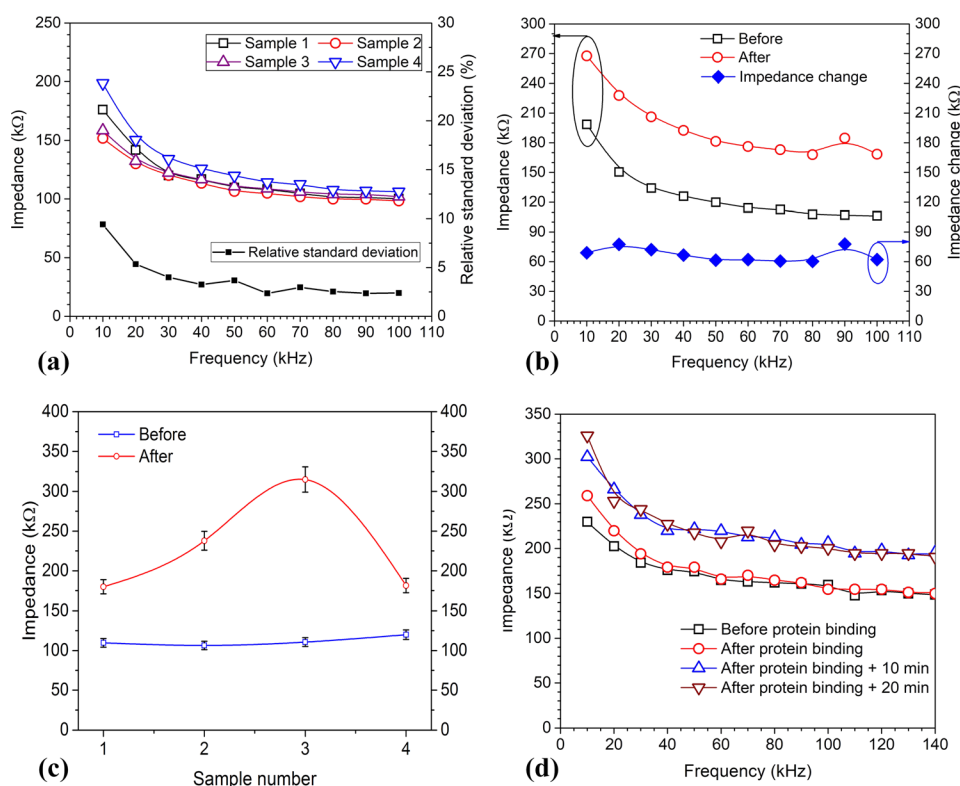


FIG. 5. Impedance of the biosensor before and after protein injection in the detection region. (a) Impedance profiles of four biochips after antibody immobilization on the electrode surface (the amplitude of the exciting signal is 600 mV_{p-p}). (b) Presence of targeted proteins confirmed by the impedance change before and after protein injection and incubation in 20 min (measured with an amplitude of the exciting signal of 600 mV_{p-p}). (c) Impedance change measured at 50 kHz and 600 mV_{p-p} amplitude of the exciting signal for four prototypes before and after protein injection and incubation in 20 min. Distinguishable lines confirm the presence of the targeted protein. (d) Impedance versus time used to investigate proteins captured on electrodes in the case where 100 mV_{p-p} amplitude of the exciting signal is applied. Profiles were captured before and after protein immobilization and after 10 and 20 min after proteins were trapped on electrodes.

and after the protein immobilization process. The impedance values for the chips are the same at high frequencies, where the impedance is mainly based on the two-layer capacitor. At low frequencies, where the surface resistivity dominates the impedance results, there is a small deviation between chips. Figure 5(b) shows that the impedance of the sensing electrode increased after protein injection and incubation in 20 min with an increase in the range of 60–77 $k\Omega$. The impedance changes before and after protein injection and incubation in 20 min of the four biochip prototypes have the same trends. The impedance values of the sensing elements of the four prototypes measured at a frequency of 50 kHz are shown in Fig. 5(c). Changes in the resistance are up to 51.3%.

C. Impedance measurement with time

The change in impedance with time was also recorded; the results are shown in Fig. 5(d). Four measurements were conducted before and after protein trapping. An exciting signal with an amplitude of 100 mV_{p-p} is utilized for the measurements. We confirmed that after proteins were trapped, the impedance increased. After washing the chip with PBS to ensure that only the target proteins remain on the electrode surface, the impedance increased by more than 50 $k\Omega$; for example, at 50 kHz, impedance increased from 170 to 220 $k\Omega$. Note that the measurement was implemented 10 min after washing the chip. The measurement results showed that the impedance value remained unchanged with time; the value measured at 20 min was almost the same as that measured at 10 min [Fig. 5(d)]. This result confirms the reliability of the measurement.

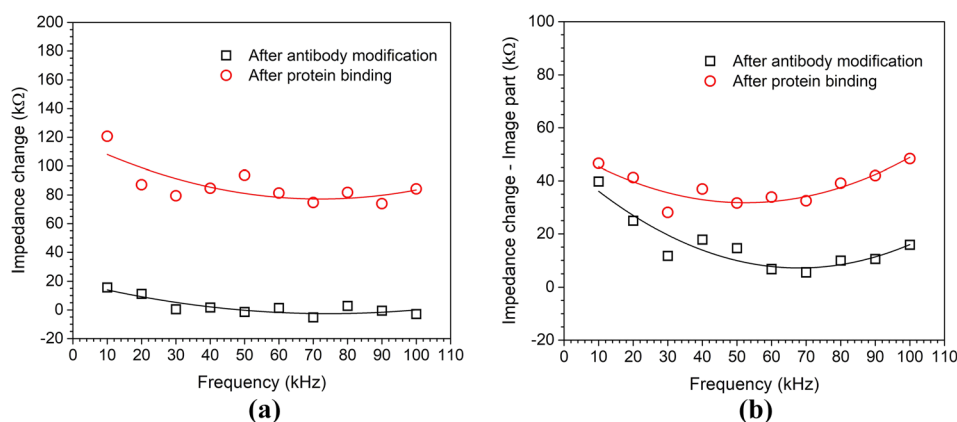


FIG. 6. Impedance change during antibody binding and protein trapping on gold electrodes. (a) Difference in the impedance change of the biochip before and after antibody growth on gold electrodes and after protein preconcentration. (b) Change in the imaginary part of biosensor impedance after antibody binding on gold electrodes and after protein trapping by antibodies.

The presence of antibodies and trapped proteins was determined using the impedance measurement module, which measures the change in both the imaginary and real parts of impedance. First, the impedance of the biochip with only PBS solution fully filling the microchannel was measured for reference. Then, the impedances of the sensing element after the antibodies had become immobilized on the gold electrodes and after proteins had become trapped on gold electrodes were measured. The impedance was then measured 10 min after the microfluidic channel was cleaned and fully filled with PBS solution. Figure 6(a) shows the change in impedance at multiple frequencies. The presence of proteins can be determined from the total impedance change. The total impedance of the biosensor increased by approximately 80 $k\Omega$ after protein injection and trapping. In the imaginary part, the results show the separated imaginary impedance curves when no antibody on the gold electrode is present, when there is an antibody binding on electrodes, and when there is a trapped protein present on gold electrodes [Fig. 6(b)]. The imaginary part of impedance increased by about 20 $k\Omega$ at 50 kHz after antibodies bound to the gold electrodes and then further increased by about 20 $k\Omega$ at 40 kHz when proteins were trapped by antibodies.

The imaginary part of impedance was used for impedance measurement analysis. The series capacitance that changes the imaginary part of impedance was calculated, as shown in

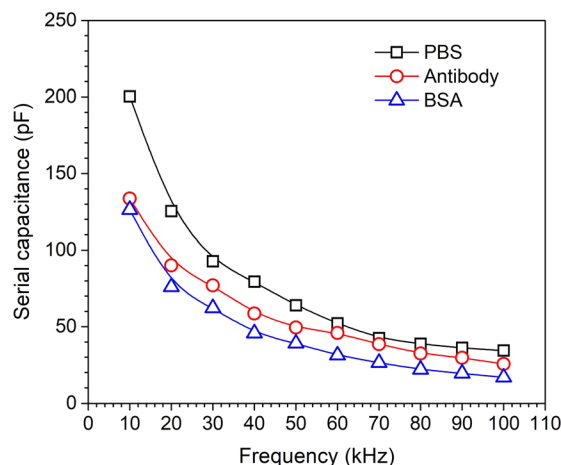


FIG. 7. Serial capacitance of biosensor at multiple frequencies when PBS solution fully filled the channel, antibodies were on electrodes, and proteins were trapped.

Fig. 7. The three separated lines investigated at multiple frequencies are observed before and after antibody growth and after protein trapping on electrodes.

In Eq. (2), C_s is a function of frequency, C_f is the surface capacitance, and R_f is the surface resistance. At high frequencies, the series capacitance between electrodes is below 34, 26, and 16 pF for C_s at 100 kHz with PBS solution, antibodies bound on gold electrodes, and trapped proteins, respectively. The impedance measurement results show the presence of antibodies grown on gold electrodes and proteins trapped by antibodies. Both the capacitance and resistance are changed by the presence of proteins and antibodies on gold electrodes. The impedance changed from 50 to 100 k Ω after proteins were trapped on electrodes by antibodies. The series capacitance of this model indicates the presence of antibodies growing on gold electrodes with the separated line impedance profile measured at multiple frequencies. The amplitude of the exciting signal was changed from 600 mV_{p-p} to 100 mV_{p-p} in the measurements to investigate the effect of AC voltage on the measurement results. Through the obtained results on evaluating the performance of the impedance sensor to detect 10 μ M BSA which is statically incubated for 20 min, we could confirm the feasibility of using the impedance measurement approach for the quantitatively measurement of protein concentration.

V. CONCLUSION

In this work, a low-cost, compact biochip was designed and fabricated for protein detection. Nanofractures created by gold nanoparticles are used for ion enrichment and depletion to form a trapping zone when electroosmotic flow occurs in the microchannel. An impedance measurement module was implemented based on the lock-in amplifier technique to measure the impedance change during antibody growth on the gold electrodes and that was caused by trapped proteins in the detection region. The impedance measurement results indicate the presence of trapped proteins. Distinguishable impedance profiles, measured at multiple frequencies, taken before and after the presence of proteins in the detection area were obtained, confirming the effectiveness of the proposed system. Although the concentration performance by the impedance measurement is potential, further experiments for the quantitative measurement could be the future direction to study.

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