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A Smart Bioelectrocatalytic Immunosensing Lab-on-a-chip for Portable Diagnostic Application

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Abstract

This paper presents a smart bioelectrocatalytic immunosensing lab-on-a-chip that uses a novel electrochemical method and can be applied to portable diagnostic systems. Fluid flow in this biochip is induced by capillary attraction force and is controlled by geometrical structure and surface modifications. With this fluid transport mechanism, the biochip does not require external electric power or control parts. Therefore, it is suitable for disposable lab-on-a-chip applications. The fabricated biochip consists of two Polydimethyl-siloxane (PDMS) layers and glass substrate with immunoelectrodes and is $25 \times 20 \times 6\ mm^3$ in size.

Keywords: lab-on-a-chip, Immunosensing, Bioelectrocatalysis, Capillary attraction force, Surface tension

Introduction

The development of lab-on-a-chip (LOC) using immunoassay has evolved to include applications in clinical diagnostics. The studies for highly-sensitive and reliable immunosensing biochips integrated with biospecific electrodes are currently expanding with the union of bioanalytical science and MEMS technology[1,2]. Combining these two technologies provides some synergy for speed, ease of use, and relatively low cost per sample, each of which is important for portable clinical systems[3].

For the immunosensing LOC system, researchers follow two main development approaches to design efficient signal detection strategies and for the microfluidic-based chip designs proper for sensing strategies.

In order to get the meaningful physical signals associated with the biological detection and immunoassay, various methods are employed, such as electrochemical, piezoelectric, or optical techniques. Among these, the electrochemical method is most suited for miniaturized devices because of the simple instrumentation and easy signal quantification. On the other hand, the electrochemical method also requires complicated antibody labeling steps or expensive labeled secondary antibodies.

Another focus is the development of reliable microfluidic manipulation techniques for immunosensing LOC. Various researchers have explored active control devices such as active micropumps and microvalves[4-6]. However, active microfluidic control is not acceptable in portable diagnostic systems due to high cost, difficulty in integration, complexity in fabrication and the need for an external power supply[7,8]. Instead, for disposable LOC, passive microfluidic control rather than active control is viable because the fabrication is simple and no external power is required.

This paper presents a smart PDMS-glass disposable biochip driven by capillary attraction. Smart fluid control is achieved by geometrical structure and hydrophobic patterns without any external power source. The difference in fluidic resistances caused by the difference in width in the channel junctions acts as the valve so that fluid flow is controlled without additional valving components. Therefore, the designed immmunosensing biochip is suitable for single-use LOC applications.

The immunosensing mechanism in this biochip is electrochemical signaling from an antigen-antibody interaction with bioelectrocatalyzed enzymatic signal amplification[9]. The proposed signaling strategy is based on the back-filling method and uses enzyme-labeled antibody on the immunosensing surface along with biocatalytic enzyme and does not require complicated antibody labeling steps or any labeled secondary antibody[10].

Design

The key design point of the immunosensing LOC is that its operation does not depend on an external power supply and is adaptable for handheld or portable diagnostic systems. The designed biochip does

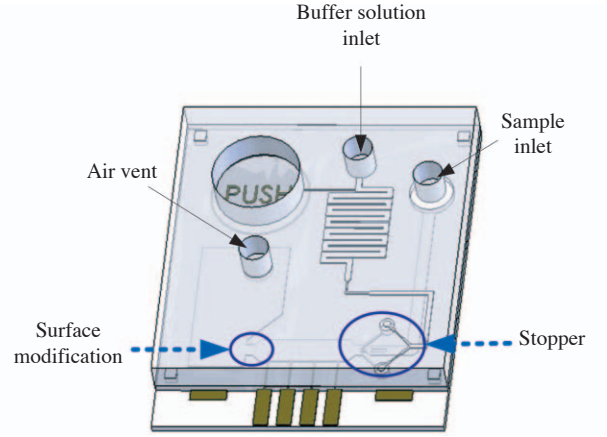

Figure 1. Schematic sketch of the immunsensing biochip design.

not require external electric power or contain internal moving parts such as actuator diaphragms and microvalves that are used in active microfluidic systems. To accomplish this, we used capillary attraction force as the sample loading force and surface tension as the flow valves at points where channels suddenly expand. Figure 1 shows the schematic sketch of the biochip design. The analyte sample is filled automatically by capillary attraction force and controlled by a geometrical stopper and surface modification.

In the analyte sample washing process, pneumatic pressure in the air bladder drives the buffer solution into the immunosensing chamber. When pneumatic pressure is applied, bubbles remaining in the interconnection holes are shifted into the inlet region and captured by surface tension at the poles. The captured bubbles stop the flow of the buffer solution into the

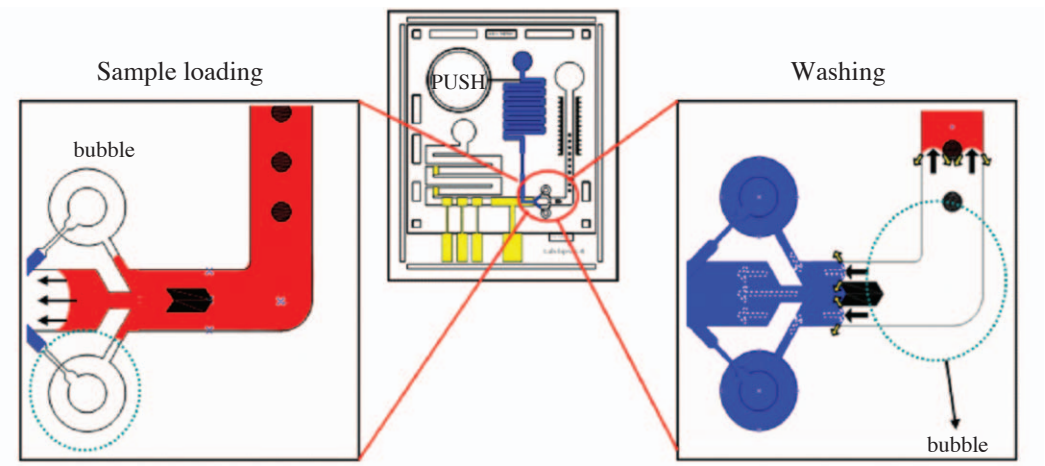

Figure 2. Operating mechanism of the designed immunosensing biochip.

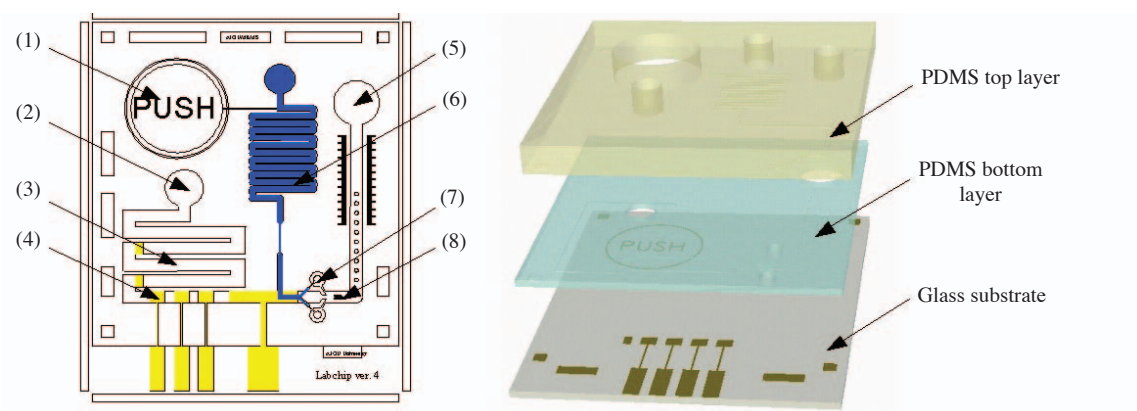

Figure 3. Schematic illustration of the designed biochip structure. Left side is top view of the designed biochip. (1) air bladder, (2) air vent, (3) waste chamber, (4) immunosensing electrodes, (5) sample inlet port, (6) buffer solution reservoir, (7) interconnection hole, (8) fluid barrier pole.

sample inlet port, so that the buffer solution flows in the direction of the immunosensing chamber and washes the analyte samples. Figure 2 show the operating mechanism of the designed immunosensing LOC.

The proposed polymer-based immunosensing biochip consists of two PDMS layers and a glass substrate with immunosensing electrodes as shown in Figure 3. The upper PDMS layer has a buffer solution reservoir and an air bladder for driving buffer solution into the immunosensing chamber. The bottom PDMS layer has passive microfluidic components including the sample inlet port, detection chamber, waste chamber and interconnection holes linked to the buffer solution reservoir. To hold the shifted bubble, 250-μm-diameter PDMS poles stand between the interconnection holes and the sample inlet port. The PDMS poles near the inlet port enhance surface tension in the inlet channel, the shifted air bubbles are captured at the PDMS poles with pneumatic pressure and the captured bubbles enhance the fluidic resistance of the inlet channel. Most buffer solution flows into the immunosensing chamber due to the air bubble barrier formed during the washing process. The channel width and depth are 800 μm and 120 μm, respectively.

Other important issues in the development of immunoassays are the immunosensing interface and signaling strategy. In this study, we used an electrochemical immunosensing strategy in which the sensor signal is amplified by an enzymatic catalytic reaction at the interface. This signal amplification strategy does not require the cumbersome process of antibody labeling or the use of a labeled secondary antibody. For affinity sensing, non-labeled native antibody and periodate-treated glucose oxidase (GOx) are reacted and biospecifically bound to the functionalized antigen or ligand group. The remaining surface amine groups on the dendrimer layer are covalently covered with GOx ("back-filled"). From the bioelectrocatalytic reaction with immobilized GOx and the electrochemical signal registration, the surface density of biospecifically bound antibody can be quantified. As a model antigen functionality, the 2, 4-dinitrophenyl (DNP) group was immobilized on the gold electrode surface using a poly (amidoamine) dendrimer layer as the interfacing monolayer. Anti-DNP antibody was used as the target molecule in affinity biosensing. The dendrimer-assisted immunosensing interface has the merits of an organized monolayer and the ligand functionalization with high density and molecular accessibility. As shown in Figure 4, the proposed signaling strategy is based on back-filling the immunosensing surface with a biocatalytic enzyme, GOx.

Results and Discussion

Flow Characterization

Fluid flow tests of the fabricated biochip were conducted according to the back-filling immunosensing strategy. Figure 7 shows the operating process of the immunosensing biochip. The operation procedure

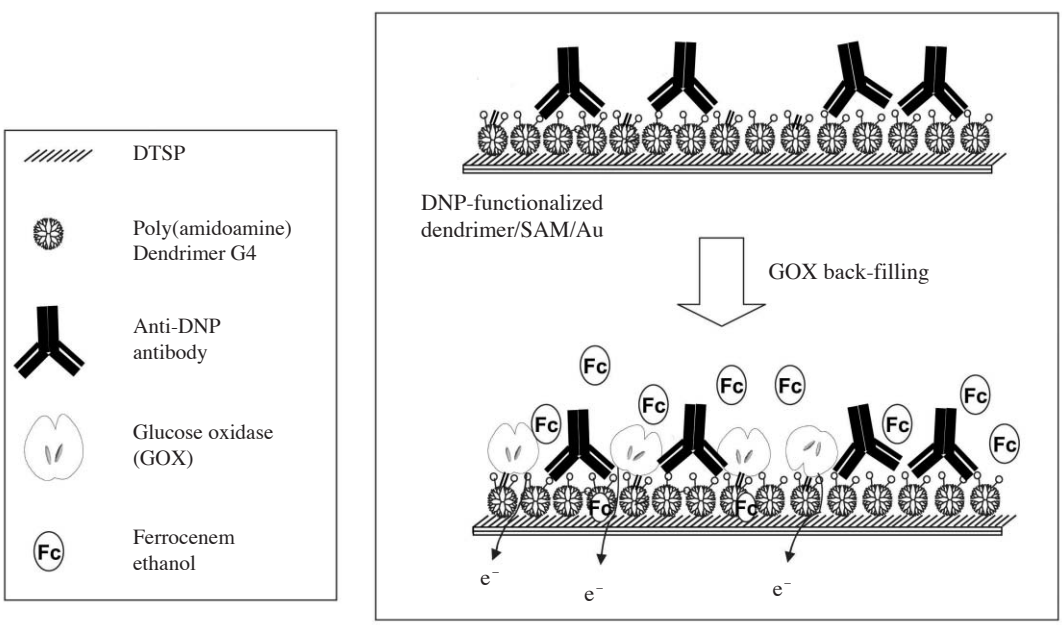

Figure 4. Schematic representation of the electrochemical immunosensor with the enzyme (GOx) and bioelectrocatalysis.

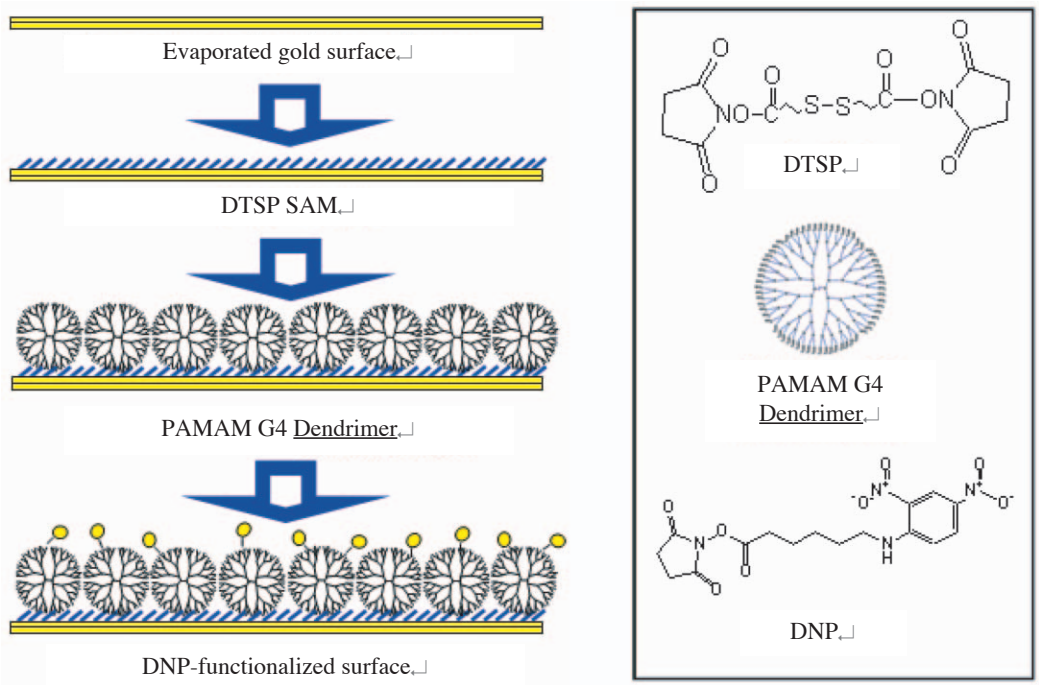

Figure 5. Schematic representation of the fabrication procedure of the immunoelectrodes.

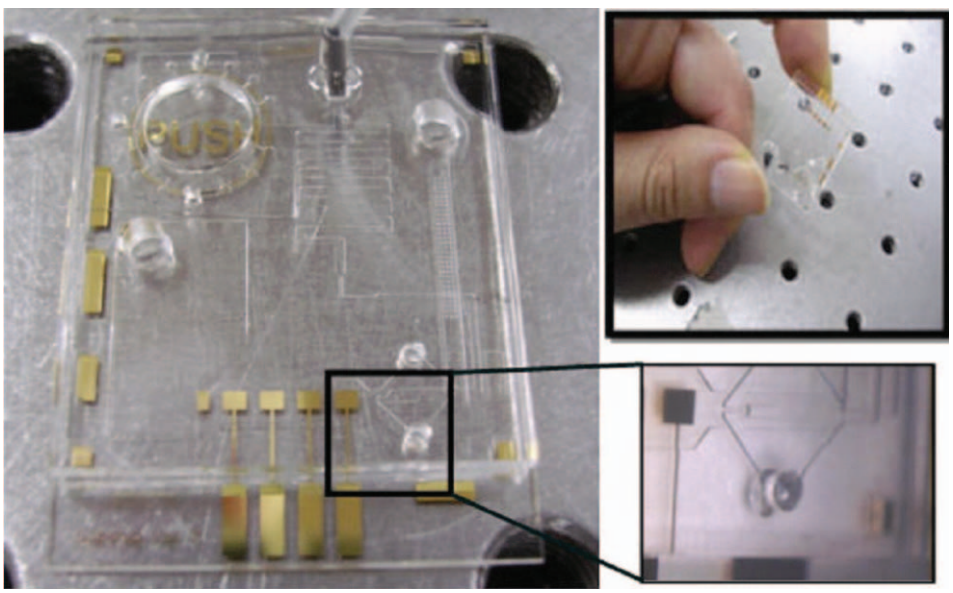

Figure 6. Photograph of the fabricated biochip and interconnection structure.

involved the buffer solution loading step, sample reaction step and the sample washing step with the buffer solution. The buffer solution, which contained PBS with 0.1 mM ferrocene-methanol and 10 mM glucose, was loaded from the buffer solution inlet port to the interconnection holes. The sample including the antibodies (anti-DNP) and the activated enzyme (GOx) was loaded autonomously by capillary attraction force from the sample inlet port to the immunosensing chamber where it reacted with the antigen-functionalized electrodes. After the antibodies and the immobilized antigens reacted for 30 min, pneumatic pressure was applied at the air bladder to begin the washing process. The sample in the detec-

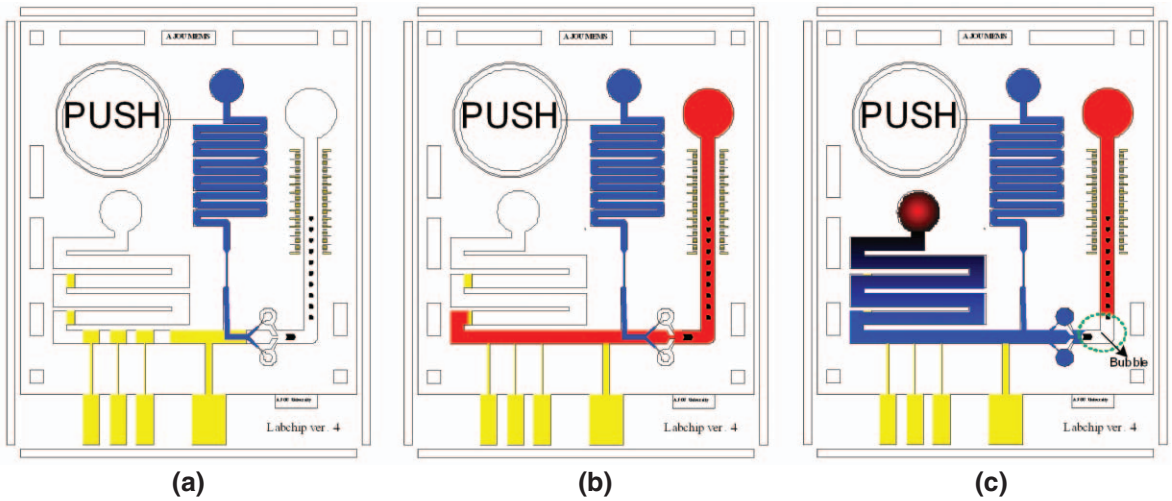

Figure 7. Operating process for the biochip. (a) buffer solution loading, (b) sample reaction, (c) washing with buffer solution

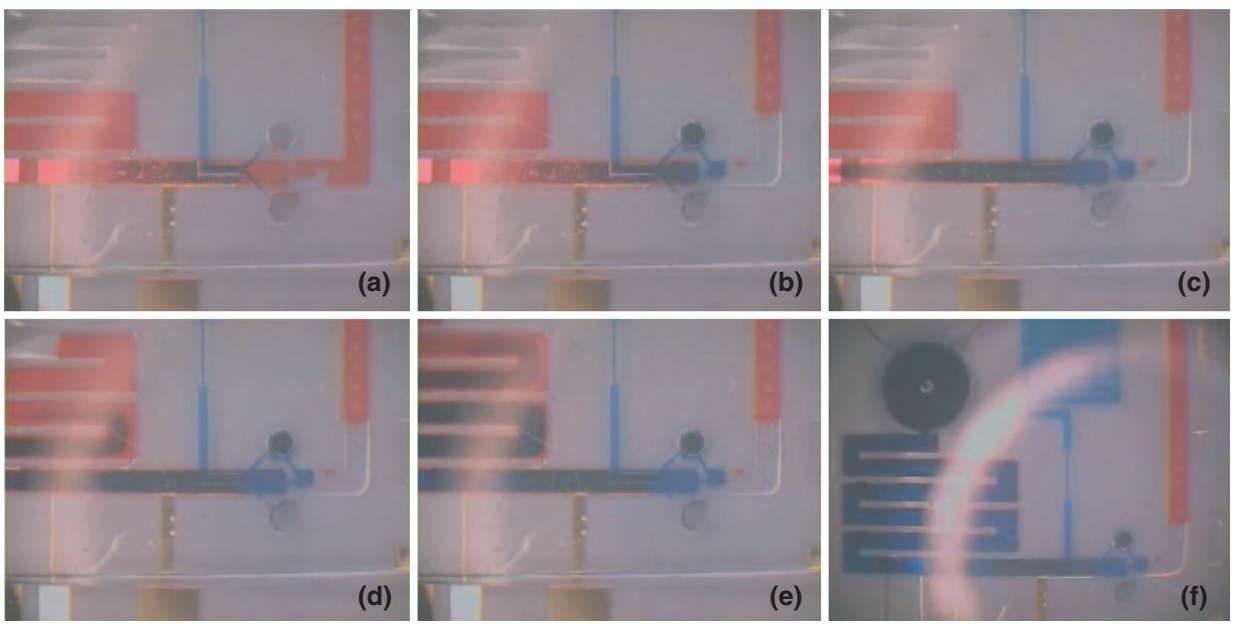

Figure 8. Captured video clips showing the sequential sample washing process. (a) the sample reaction, (b-f) clips show the sequential washing process with buffer solution (every 2 seconds)

tion chamber was washed out through the interconnection holes with buffer solution containing the electron transferring mediator.

Figure 8 shows sequential video clips captured during the sample washing process. In the washing process, shifted bubbles are observed trapped at the PDMS poles near the inlet port. The trapped bubbles dammed the backward flow of buffer solution into the inlet port and pushed the solution into the immunosensing chamber. The results show that, as designed, the passive components and their operating mechanism are effective for reliable fluidic control without any active moving parts.

Electrochemical Immunoassay

Figure 9 shows the experimental set-up for the electrochemical immunosensor to transduce the affinity recognition reaction. Electrochemical measurements were carried out with an electrochemical analyzer model 630B (CH Instruments). To confirm the

occurrence of GOx bioelectrocatalysis at the affinity sensing electrodes, voltammetric measurements were conducted with buffer solution containing various concentrations of glucose substrate. The cyclic voltammetric trace shown in Figure 10 represents the electrochemical signal generation and amplification from the fabricated immunosensing biochip. These results confirmed the possibility of electrochemical analysis using GOx.

After confirming the possibility of signal amplification by GOx on the fabricated chip, the immunosensing test was conducted with the back-filling method. This method is based on the bioelectrocatalytic reaction with immobilized GOx whereby biospecifically immobilized antibody can be quantified as the inverse proportion of immobilized GOx at the immunosensing electrode surface. GOx was immobilized on the dendrimer surface as a signaling molecule where there is no immobilized antibody, so the immobilized GOx correlates in inverse proportion to the concentration of immobilized antibody. We can therefore confirm the concentration of immobilized antibodies in the sample by the electrochemical signal from the immobilized GOx. The test results with samples containing various concentrations of anti-DNP antibody are shown in Figure 11. When the concentration of anti-DNP antibody in the sample is high, the amount of immobilized GOx attached to the electrodes decreases, resulting in low amplification of the electrochemical signal. Thus, the collected signals directly correlate to the amount of anti-DNP antibody immobilized on the electrode surface.

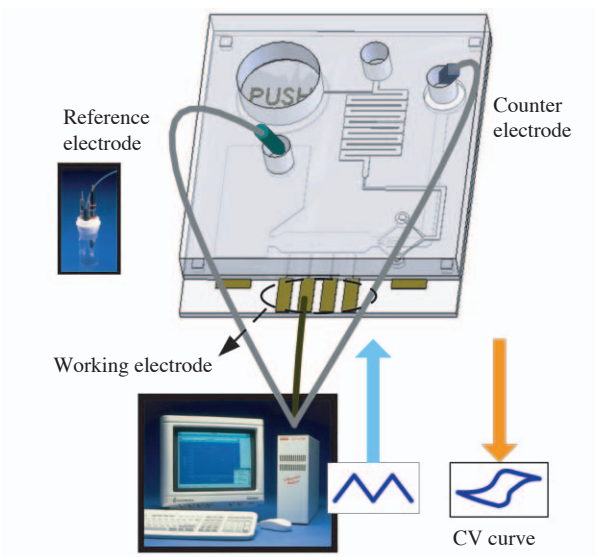

Figure 9. Schematic representation of the experimental setup.

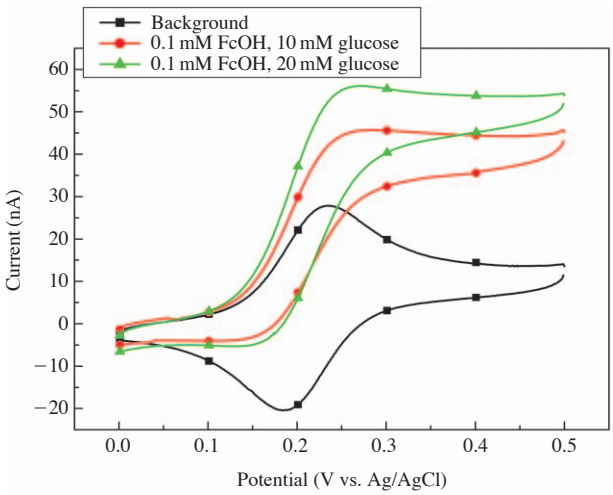

Figure 10. Cyclic voltammetric traces of the electrochemical signal generation and amplification of GOx using various concentrations of buffer solution. Voltage sweep rate is 50 mV/s.

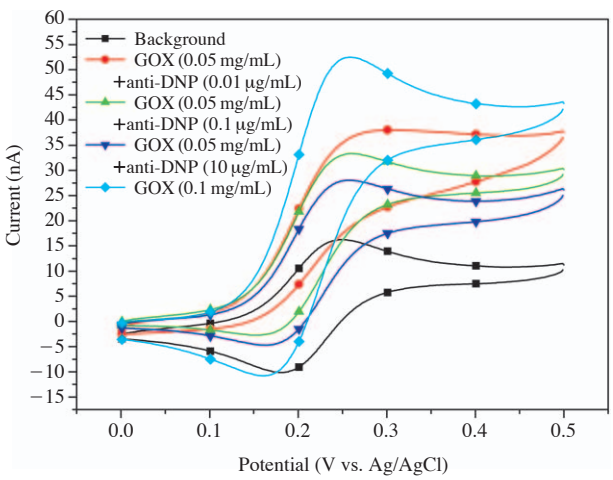

Figure 11. Cyclic voltammetric traces for DNP/anti-DNP affinity biosensors as a function of target protein concentration.

Prior to signal acquisition with the different concentrations of anti-DNP antibodies, we conducted a bioelectrocatalytic signal test of a sample that included only 0.1 mg/mL of GOx at the electrode surface to confirm the amplified current from the maximum amount of immobilized GOx. The maximum amplified signal current with GOx in the fabricated biochip registered as 43.25 nA at 400 mV, as shown in Figure 10. We obtained different amplified signals by changing the concentration of anti-DNP antibody. With the sample containing 0.01 μg/mL of anti-DNP antibody,

the amplified signal current registered as 37.16 nA at 400 mV, which was a decrease of 6.09 nA compared to the maximum current when fully covered with GOx. This result shows that immobilization of the anti-DNP antibodies on the electrode surfaces reduces the immobilization of GOx. As a result, we can measure the amount of immobilized anti-DNP antibody in the range of 0.1 μg/mL of anti-DNP antibody in the sample. This result suggests that, by changing the DNP-functionalization ratio, the sensor sensitivity and the detection range can be modulated.

Conclusions

In this paper, an electrochemical immunosensing biochip was designed, fabricated and successfully confirmed. Aside from the washing process that involves pushing the air bladder, the entire process is performed autonomously. From the experimental results, we confirmed that the enzymatic back-filling immunoassay method is simple and effective for miniaturized diagnostic systems. From the calibration tests for anti-DNP, we achieved a wide dynamic detection range for the immunosensor. In the near future, we hope to improve the performance by adapting a better structure and to develop a portable monitoring system for the disposable biochip.

Methods

Materials

Polymer is a promising material for disposable biochips. Among the available polymers, Poly (dimethylsilosans) (PDMS) is widely used as an effective material for its simple molding fabrication process. In developing an immunosensor where many designs may need to be tested, the ease of rapid prototyping with PDMS is a critical advantage.

Poly (dimethylsilosans) and its curing agent were purchased from Dow Corning (PDMS, Sylgard 184) along with Pyrex glass (#7740). The molds for the PDMS layers used negative photoresists (SU-8 2100, Microchem Corporation). 3, 3′-Dithiodipropionic acid di (N-hydroxysuccinimide ester) (DTSP), glucose oxidase (GOx, from Aspergillusniger), and h-D-glucose were purchased from Sigma. Poly (amidoamine) fourth-generation dendrimer, ferrocenemethanol, 4-nitrobenzaldehyde, and sodium periodate were obtained from Aldrich. 6-(2, 4-dinitrophenyl) aminohexanoic acid succinimidyl ester (DNP-NHS) as a ligand and anti-dinitrophenyl-KLH antibody (anti-DNP, rabbit IgG fraction) as a target protein were supplied from Molecular Probe and used without further purification. All other materials were of the highest quality available. For solutions, doubly distilled and deionized water with a specific resistance over 18 MΩ · cm was used throughout the study.

Fabrication of the Immunosensing LOC

All components of the PDMS layer were fabricated through the sequential spin-coating, soft-curing, and bonding process of PDMS elastomer. The thickness of the PMDS layer was controlled by the amount of PDMS pre-curing agent, and the irreversible bonding process was used for PDMS-to-PDMS and PDMS-to-Glass bonding.

To create the PDMS layers, liquid PDMS prepolymer (a mixture of 1 : 10 base polymer with curing agent) was poured onto the patterned SU-8 mold to a thickness of 120 μm and cured in a 60°C oven for two hours. The cured PDMS layers were peeled off the SU-8 master and the sample inlet, buffer solution inlet, air vent and interconnection holes were punched out. The upper part of the biochip was assembled by stacking two PDMS layers after O_2 plasma treatment for 10 seconds at 100 watts. The entire PDMS layer assembly was then cured again at 60°C for 2 hours.

For the immunosensing gold electrodes on the glass substrate, Ti/Au (500Å/2500Å) were deposited onto the Pyrex glass using a sputter system and patterned using general photolithographic technology. DNP (dinitrophenyl) was immobilized on the immunosensing electrode as a possible reactant for the target molecule. Figure 5 shows a schematic representation of the procedure to construct the DNP-modified surface on the glass substrate as the immunoelectrodes. Amine-reactive SAM was formed by dipping the surface into 5 mM DTSP in DMSO for 2 hours. After washing with DMSO and ethanol, the electrodes were transferred to 0.5% (w/w) poly (amidoamine) G4 dendrimer solution in ethanol for 30 minutes. Finally, antigen (DNP) was functionalized on the dendrimer-modified surface.

The fabricated glass substrate, which works as the immunoelectrode, was then bonded with the PDMS layers treated with O_2 plasma. To prevent destruction of the constructed SAM layers on the electrode, the glass substrate was not treated with O_2 plasma at any time during the assembly process. Figure 6 shows the $25 \times 20 \times 6$-mm^3 fabricated biochip.

Acknowledgements

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