31st Annual International Conference of the IEEE EMBS
Minneapolis, Minnesota, USA, September 2-6, 2009

Exploiting Sub-threshold and Above-threshold Characteristics in a Silver-Enhanced Gold Nanoparticle Based Biochip

Yang Liu *, Evangelyn Alocilja †, Shantanu Chakrabartty *
liuyang4@egr.msu.edu, alocilja@egr.msu.edu, shantanu@msu.edu
*Department of Electrical and Computer Engineering
†Department of Biosystems Engineering Michigan State University
East Lansing, MI 48824 USA

***Abstract*—Silver-enhanced labeling is a technique used in immunochromatographic assays for improving the sensitivity of pathogen detection. In this paper, we employ the silver enhancement approach for constructing a biomolecular transistor that uses a high-density interdigitated electrode to detect rabbit IgG. We show that the response of the biomolecular transistor comprises of: (a) a sub-threshold region where the conductance change is an exponential function of the enhancement time and; (b) an above-threshold region where the conductance change is a linear function with respect to the enhancement time. By exploiting both these regions of operation, it is shown that the silver enhancing time is a relaible indicator of the IgG concentration. The method provides a relatively straightforward alternative to biomolecular signal amplification techniques. The measured results using a biochip prototype fabricated in silicon show that 240 pg/mL rabbit IgG can be detected at the silver enhancing time of 42 min. Also, the biomolecular transistor is compatible with silicon based processing making it ideal for designing integrated CMOS biosensors.**

***Index Terms*—Gold nanoparticle, silver enhancement, biochip, biomolecular transistor**

I. Introduction

Biosensors are important analytical tools for detecting and controlling disease outbreaks, which according to the United States Department of Agriculture (USDA) cause $2.9-$6.7 billion worth of losses every year [1]. Biosensors can be differentiated from each other based on the type of probes and detection methods employed on the device. For instance, enzyme-linked immunosorbent assay (ELISA) is one of the oldest and most widely used laboratory methods and uses optical detection. It yields a colorimetric signal upon enzymatic cleavage of chemiluminescent substrate and has detection limits approaching picomolar range. Another popular method for immunoassays is based on gold nanoparticles which are used as labels and signal generators. However, the sensitivity of this approach is limited by resolution of the optical detector especially when the concentration of gold nanoparticles is very low [2]. The sensitivity of the detection, however, can be increased significantly by using a procedure called "silver enhancement" where silver deposition occurs around nucleation sites formed by the gold nanoparticles. Some examples of immunoassays that use gold nanoparticles in conjunction with silver enhancement can be found in [2], [3], [4], [5]. For instance, in [3] gold nanoparticle and silver enhancer solution have been employed in the immunoassay for detecting ricin with a detection limit of 100 pg/mL.

However, biosensors with electrical readouts offer several advantages over their optical counterparts due to their reduced cost, reduced form factor and the ease of signal acquisition [6], [7]. One of the major challenges in the electrical or impedance based detection is low signal-to-noise ratio as compared to optical detection, which is attributed to the large magnitude of the background signal [7]. Thus, performing signal enhancement at the biomolecular level before performing electrical read-out would reduce the effects of background interference.

In this paper, we present a conductimetric biosensor that utilizes silver enhancement for biomolecular signal amplification. At the core of the biosensor is a transistor that is constructed using functionalized gold nanoparticles on the high-density interdigitated microelectrode array. The interdigitated electrodes provide a large active area to facilitate binding between the analyte and the detection probe and hence have several advantages over non-interdigitated electrode arrays [4], [8]. In this paper, we show that the silver enhancing time provides quantitative means of determining the concentration of gold nanoparticles. The salient feature of this work includes: (a) a simple and robust electrical detection method using a combination of gold nanoparticle labels and silver amplification technique; (b) characterization of the extent to which the nanoparticle adsorption can be quantified using silver enhancement; (c) characterization of two distinct biomolecular transistor responses that are the sub-threshold and the above-threshold regions of the operation and (c) characterization of the biochip sensitivity and the detection limit using repeated and controlled experiments.

This paper is organized as follows: Section II describes the operating principle of the silver enhancement technique when applied to gold nanoparticles and the high-density microelectrode biochip. Section III describes the fabrication method of biochips and surface functionalization of biochips. Section IV presents experimental results of detecting rabbit IgG, which verify the principle of silver enhancement and the functionality of the biochip. Section V concludes with a brief discussion and the future work.

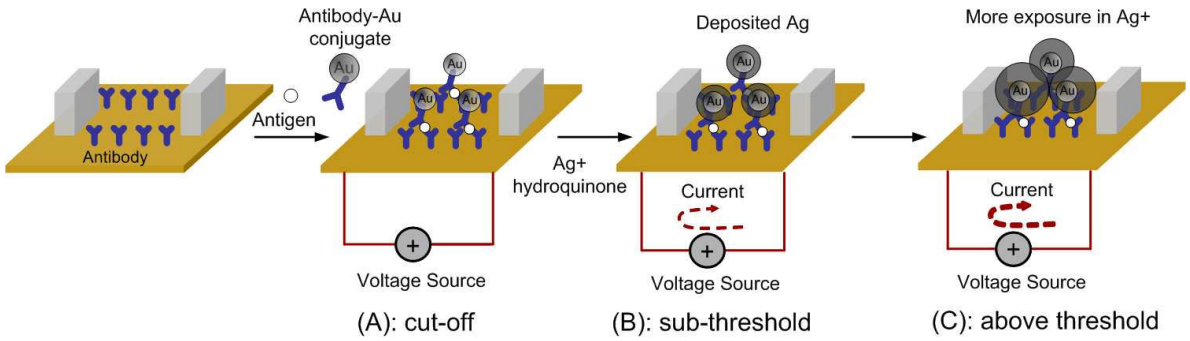

Fig. 1. The operating principle of the silver-enhanced biomolecular transistor with three distinct regions of the operation.

II. Principle of Silver Enhancement using Gold Nanoparticles

The principle of conductimetric detection is shown in Fig. 1 where initially probes specific to the target molecules are immobilized in the regions between two electrodes. When the analyte is applied, the target biomolecules hybridize with the specific probes. The secondary antibodies conjugated with gold (Au) nanoparticles are then applied to the biochip, which leads to the formation of a sandwich array as shown in Fig. 1(A). This configuration is denoted as the "cut-off" region since the current measured between the electrodes (for a fixed potential difference) is small. In the next step of the silver enhancement procedure, the active component (with gold nanoparticles) of the biochip is exposed to a solution of Ag(I) and hydroquinone (photographic developing solution). The gold nanoparticles act as a catalyst and reduce silver ions into metallic silver in the presence of a reducing agent (hydroquinone). The reduced silver then deposits on the gold surface, thus enlarging the size of the gold nanoparticles. As the size of the silver islands grow, they provide shorter paths for electrons to hop between the electodes. The region of operation when the distance between the elecrodes has not been fully bridged by the silver islands is the sub-threshold region (see Fig.1 (B)). With the increase in enhancement time, the consistent growth of silver-enhanced particles completely bridges the area between the electrodes. Under this condition, the device enters the above threshold region of operation where a flow of current can be measured when a fixed potential is applied between the electrodes (Fig.1 (C)). The time required for the device to reach the threshold from the cut-off region is known as the transition time. In this paper, we use the sub-threshold and above-threshold characteristics of the device for conductimetric measurement of the concentration of the target biomolecules.

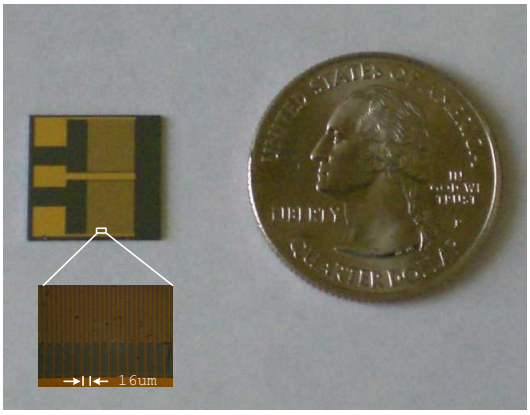

Fig. 2. The high-density interdigitated microelectrode biochip.

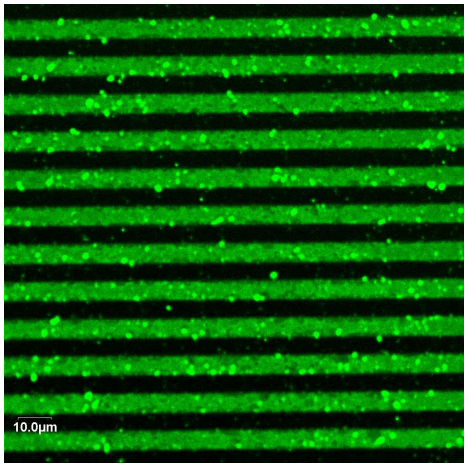

Fig. 3. Confocal laser scanning microscopy image of electrodes showing FITC-labeled bovine IgG only immobilized to silicon dioxide surfaces (black areas are the electrodes where no IgG are present).

III. Biochip Fabrication and Surface Functionalization

The biochips were fabricated from 4″ silicon wafers (p-type 100, thickness 500-550 μm). A 2 μm thick layer of thermal oxide was grown over the silicon to serve as an insulator between the electrodes and the substrate. Photolithography was used to pattern photoresist, metal electrodes were deposited by the evaporation of 10 nm of chrome under 100 nm of gold and a lift-off process was used to develop the interdigitated electrode array. Fig. 2 shows the high-density interdigitated electrodes biochip fabricated using a standard MEMS technology. Each electrode finger has a length of 5000 μm, a width of 5 μm

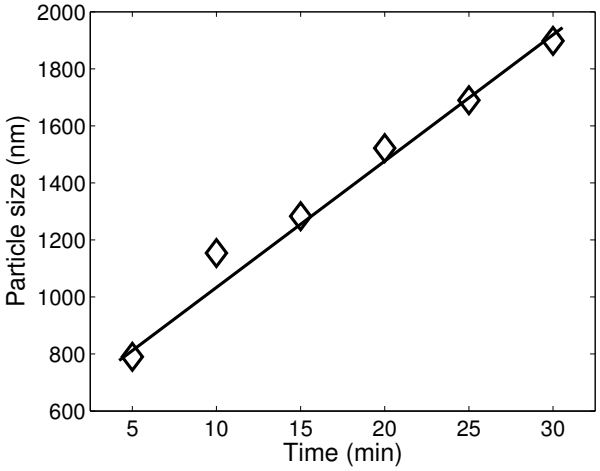

Fig. 4. The relationship between the silver-enhanced gold particle size and the silver enhancing time.

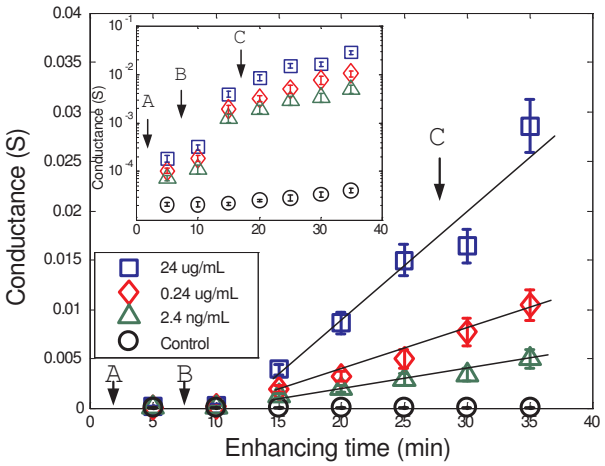

Fig. 5. Resistance of the biochips (with different target rabbit IgG concentrations) measured as a function of silver enhancing time. (Insert) Conductance measurement in logarithmic domain, which can clearly show that the gold antiparticles experienced 3 different stages (labeled as A,B, and C) to form the bridge across electrodes. A: cut-off region; B: sub-threshold region where the conductance change is an exponential function of the enhancement time; C: above-threshold region where the conductance change is a linear function with respect to the enhancement time.

and an inter-electrode spacing of 6 μm.

The surface of biochips was then modified for immobilizing the antibody. The chips were first immersed in acetone in a crystallizing dish for 10 min to dissolve away the protective PR layer. The chips were then treated with 1:1 mixture of concentrated methonal and hydrochloric acid for 30 min followed by immersion into boiling distilled water for 30 min. The biochips were allowed to air dry completely. The cleaning and drying of the biochips are now ready for silanization where it occurred in an anaerobic glove box. The biochips were immersed in a crystallizing dish containing a solution of 2 % 3-Mercaptopropytrimethyloxysilane (MTS) (Sigma; St.Louis, MS) for 2 h. The chips were then rinsed in toluene and allowed to dry completely. After silanization, N-y-maleimidobutyryloxy succinimide ester (GMBS) (Sigma; St.Louis, MA) was choosen as crosslinkers to avoid multi-protein complex [9]. The crosslinking reagent was dissolved in a minimum amount of dimethylformamide (DMF) and then diluted with ethanol to a final concentration of 2 mM. The silanized substrate was treated with crosslinker for 1 h and washed in phosphate buffered saline (PBS, pH 7.4). After the application of the crosslinker, goat anti-rabbit IgG (Sigma; St.Louis, MA) was immobilized onto the biochip active surface. The biochips were placed in a petri dish, sealed with parafilm and allowed to incubate at 37^oC for 1 h. The biochips were then treated with 2 mg/mL bovine serum albumin (BSA) (Sigma; St.Louis, MA) for 45 min. After incubation, the biochip surface was rinsed with PBS (pH 7.4) and allowed to air dry. The confocal laser scanning microscopy image was used to validate antibody immobilization and determine where it occurred (Fig. 3). The image shows that the antibody immobilization was only occurring on the silicon dioxide area between the electrodes, thus proving the effectiveness of the surface functionalization.

IV. Experimental Results and Discussions

The first step in verifying the silver enhancement principle is to measure the size of the silver-enhanced gold nanoparticles with respect to the silver enhancing time. For this experiment, Zetasizer Nano (Malvern Instruments Ltd, UK) was used to characterize the particle size. Fig. 4 shows the linear relationship between the silver enhancing time and the size of silver enhanced gold particles. We have observed that the 40 nm gold nanoparticles will reach average size of 1.2 μm when exposed to the silver enhancer for approximately 10 min. This interesting property makes the silver-enhancement principle suitable for signal amplification in conductimetric biosensors.

Based on the principle of silver enhancement, we conduct rabbit IgG detection by first applying rabbit IgG onto the active area of the biochip allowing incubation for 1 h. Goat anti-rabbit IgG and gold conjugates were then applied and were incubated for 45 min. Excess gold conjugates were washed with PBS solution. Electrical measurements are conducted after each treatment of the biochip with the silver enhancer solution (Ted Pella, Inc.,CA) and the conductance between the electrodes was measured with a BK multimeter Model AK-2880A (Worchester, MA). Fig. 5 shows the conductance between the electrodes increases with the increasing exposure to the silver enhancer solution. In the experiments, the target rabbit IgG with different concentrations are applied to the rabbit IgG biochips and similar behavior was observed. During the sub-threshold region (labeled as B) of the operation, the increase in conductance is exponential with respect to the enhancement time. This behavior is similar to the response observed in metal-oxide-semiconductor (MOS) transistors where below the threshold voltage the conductance is exponential

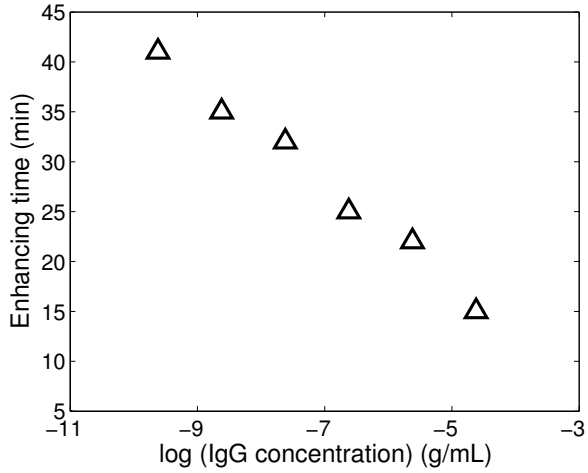

Fig. 6. Quantitative analysis: the silver enhancing time required to reach a conductance range of $3.8 - 5$ mS as a function of gold particle concentrations.

with respect to the gate-to-source potential. The similarity can be explained due to the Boltzmann transport which is common for sub-threshold conduction. It can also be seen in Fig. 5, that after the biomolecular transistor enters the above-threshold regime (labeled as C), the measured conductance is approximately linear with respect to the enhancement time. This behavior is also similar to the response observed in MOS transistors biased above threshold. Biovine IgG biochips were used as negative control experiments and we have observed that the conductance of "control" biochip start to increase at 20 min. It means that the non-specific binding actually occurred but the number of such events is much smaller than the number of specific binding events. Thus, the biochip is able to detect target IgG in the presence of background noise. We have seen that the measurement results are stable several days after the experiments have been conducted.

Some researchers have argued that the conductivity-based silver-enhanced detection is not applicable to quantitative concentration assays because the electrodes are short-circuited above a certain density of the silver-enhanced gold particles [4]. In the next experiment we will show that the quantitative analysis can be achieved by adjusting silver enhancing time. Fig. 6 shows the relation of the silver enhancing time required to reach a conductance range of $3.8-5$ mS as a function of gold particle concentration. It is interesting to note that 240 pg/mL IgG can be detected when the conductance increases to 3.8 mS at the silver enhancing time of 42 min.

We have shown the experiments to verify the principle of silver-enhanced electrical detection of rabbit IgG. One issue that other researchers have not addressed in the silver enhancement method is the accuracy and possible false positive errors. Due to the sensitivity of the presence of gold nanoparticles when exposing to silver, it might have a high level of false positive results. The typical method of prevention is to extensively wash the biochips to alleviate non-specific binding. However, the method is time-consuming and it is not always effective. Another alterative solution is to embed the biochip with error-correction function by employing encode-decoding scheme similar to the approach that we have previously reported [10].

V. Conclusion

In this paper, we have designed and characterized a silver-enhancement technique for amplifying the conductimetric measurements obtained using a high-density microelectrode array biochip. The gold nanoparticles serve as nucleation sites about which a reduction reaction deposits silver and hence enlarges the size of the gold nanoparticle. Using silver enhancer solution, the gold antiparticles can grow into a micro-size particle and ultimately can bridge the gap between electrodes, leading a measurable change in conductance. Comprehensive experiments have verified the effectiveness of surface functionalization and the functionality of biochip. The proposed biochip in conjunction with silver enhancement provides a simple, effective, and sensitive way of detecting trace quantity of pathogens.

Acknowledgment

This work is supported in part by a research grant from the National Science Foundation: NSF ECCS-0622056. Authors also would like to thank Lurie Nanofabrication Facility at University of Michigan for the fabrication of biochips.

References

[1] Food and Drug Administration, "Bacteriological Analytical Manual. 8th ed., " Arlington, VA: Association of Analytical Chemists, 2000.

[2] S. Gupta, S. Huda, P. K. Kilpatrick, and O. D. Velev, "Characterization and Optimization of Gold Nanoparticle-Based Silver-Enhanced Immunoassays," *Anal. Chem*, Vol. 79, No. 10, pp. 3810-3820, 2007.

[3] R. H. Shyu, H. F. Shyu, H. W. Liu, S. S. Tang, "Colloidal Gold-based Immunochromatographic Assay for Detection of Ricin," *Toxicon*, Vol. 40, No. 3, pp. 255-258, 2002.

[4] O. D. Velev and E. W. Kaler, "In Situ Assembly of Colloidal Particles into Miniaturized Biosensors, *Langmuir*, Vol. 15, No. 11, pp. 3693-3698, 1999.

[5] K. T. Liao and H. J. Huang, "Femtomolar Immunoassay based on Coupling Gold Nanoparticle Enlargement with Square Wave Stripping Voltammetry," *Analytica chimica acta*, Vol. 538, No. 1-2, pp. 159-164, 2005.

[6] Y. Liu, A. Gore, S. Chakrabartty, E. C. Alocilja, "A Molecular Biowire based Multi-array Biosensor with Integrated Potentiostat," *IEEE Biomedical Circuits and Systems Conference*, pp. 29-32, Montreal, Canada, 2007.

[7] J. -H. Kim, J. -H. Cho, S. C. Geun, C. -W. Lee, H. -B. Kim, S. -H. Peak, "Conductimetric Membrane Strip Immunosensor with Polyaniline-bound Gold Colloids as Signal Generator," *Biosensors and Bioelectronics*, Vol. 14, No. 12, pp. 907-915, 2000.

[8] S. J. Park, T. A. Taton, C. A. Mirkindagger, "Array-Based Electrical Detection of DNA with Nanoparticle Probes, *Science*, Vol. 295, No. 5559, pp. 1503-1506, 2002.

[9] S. K. Bhatia, L. C. Shriver-Lake, K. J. Prior, J. H. Georger, J. M. Calvert, R. Bredehorst, and F. S. Ligler, "Use of Thiol-terminal Silanes and Heterobifunctional Crosslinkers for Immobilization of Antibodies on Silica Surfaces," *Analytical Biochemistry*, Vol. 178, Issue 2, pp. 408-413, 1989.

[10] Y. Liu, S. Chakrabartty, and E. C. Alcilja, "Fundamental Building Blocks for Molecular Bio-wire based Forward-error Correcting Biosensors," Nanotechnology, vol. 18, No. 42, pp. 424017(6pp), October 24, 2007.