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Application of Nanobiotechnology to Construct a Piezoelectric Sensor Matrix Sensing the Flexibility of Immunoglobulin

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***Abstract* — We have constructed a piezoelectric sensor matrix with the capability of measuring the flexibility of immunoglobulin. The matrix consisted of 6 independent quartz crystal microbalance (QCM) coated with gold nanoparticles (GNP) of 3.5, 5, 12, 17, and 37 nm, and BSA. The detection was performed simultaneously. Antiserum against GNP bound to QCMs coated with 3.5 nm GNP and maximized for 5 nm GNP-coated QCM. To our best knowledge, this is the first biosensor sensing the topological change and flexibility of biological macromolecule.**

Keywords — Immunoglobulin, Quartz Crystal Microbalance, Matrix, Biosensor, Flexibility

I. INTRODUCTION

Surface chemistry and structure play central roles in the bio-surface interaction. Surface chemistry contributes in a major part to the binding affinity. Physical structure contributes only in a minor part to the affinity. However, structure is the major factor determines the specificity of biological binding reaction. The detection of most biosensors developed at the present time is based on both properties or solely on chemistry. Sensors that distinguish the difference in structure have yet to be developed.

Antibody-antigen recognition is commonly applied in biosensing devises for the detection of specific antigens such as protein or virus. Based on the highly specific antigen-antibody recognition, the obtained signal is proportional to the quantity of detectant bound to the coated antibody. However, it is possible to obtain the information regarding the quality of antibody applying sensor matrix coated with literally varying antigens.

We have isolated antibodies that bind specifically to gold nanoparticles [1]. The binding affinity maximizes for 5 nm GNP, reduces for 3.5 nm GNP, but drops to none for GNPs larger than 8 nm. Apparently, the flexibility of Fab-hinges of the immunoglobulin is limited [2-4]. The restricted flexibility allows only a range of antigen-antibody recognition although the surface chemistry is comparable.

A quartz crystal microbalance (QCM) is a mass-detection device that operates based on the piezoelectric properties of quartz crystals. The QCM is unsophisticated, cost-effective, real-time responding, high-resolution and stable. Because of their extraordinary sensitivity and stability, QCMs have been applied in recent years as biosensors for the online detection of biomolecules [5, 6].

The purpose of this study is based on the differential binding affinity of the antibody against GNPs of different sizes. A quartz crystal microbalance matrix was constructed based on the matrix system reported previously. Each QCM was coated with a single species of GNP of specified size. This QCM matrix thus will be capable of detecting the flexibility of immunoglobulin based on the profile of frequency shift.

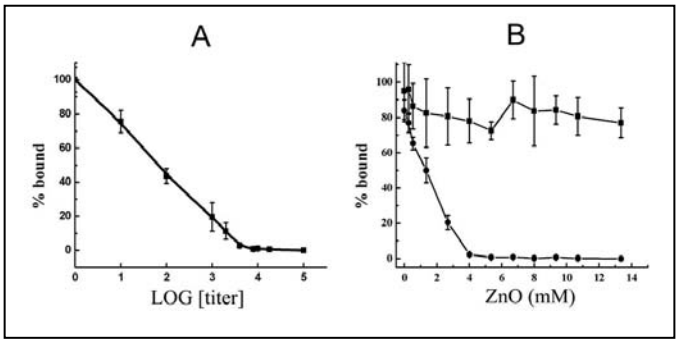

Figure 1. Titer of anti-GNP antiserum (A) and competition ELISA of antiserum-GNP binding using 5 nm GNP (circle) and 5 nm ZnO (square) as competitors (B).

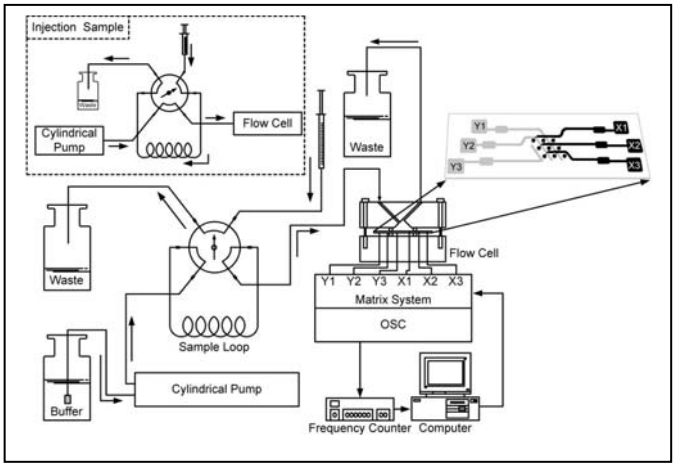

Figure 2. QCM-FIA system

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II. MATERIALS AND METHODS

A. Materials

The oscillator (Catalog#35366-10) and flow cell (Catalog#35363) were purchased from International Crystal Manufacturing Co. (Oklahoma City, USA). The QCM was fabricated from a 0.2-mm-thick AT-cut quartz wafer. A laboratory-constructed transistor–transistor logic integrated circuit (TTL-IC) was used to power the QCM. The TTL-IC was based on IC 74HC93、74LS138、74LS95、74LS04 and D1A050000 and D1C050000 relays (KUAN HIS Co.). An Agilent HP 53132 Universal Frequency Counter was used to monitor the frequency output.

B. QCM FIA system

The gold electrode was cleaned by immersion in 1.2 M NaOH for 20 min, 1.2 M HCl for 5 min, and distilled water for 5 min; after a final rinse with 95% alcohol it was air-dried at room temperature [9]. For activation, the gold electrodes were treated with 2.5% glutaraldehyde (0.5 µL) for 15 min, washed briefly with distilled water, and then dried. Two microliters of GNP (0.3 mM) was applied to the pretreated gold electrode and incubated in a humid hood for 20 min. For the preparation of control QCMs, bovine serum albumin (BSA; 1 mg/mL, 0.5 µL) was applied instead. The coated electrodes were washed thoroughly with double-distilled water, followed by a PBS wash. Blocking was achieved by adding BSA (1 mg/mL, 0.5 µL), incubating in a humid hood for 1 h, washing with water, rinsing with PBS, and then air-drying. The coated QCM was assembled in a flow cell through which PBS was passed at a flow rate of 0.1 mL/h. The frequencies of all of the QCMs were monitored until steady state conditions were achieved (usually 30 min to 1 h) [7].

C. Preparation gold nanoparticle

The seed colloids were prepared by adding 1 mL of 0.25mM $HAuCl_4$ to 90 mL of H_2O and stirred for 1 min at 25 °C [8, 9]. Two milliliters of 38.8 mM sodium citrate was added to the solution and stirred for 1 min followed by addition of 0.6 mL freshly prepared 0.1 M $NaBH_4$ in 38.8 mM sodium citrate. Different diameters of gold nanoparticles ranging from 3.5 nm to 12 nm were generated by changing the volume of seed colloid added. The solution was stirred for an additional 5 to 10 min at 0 to 4 °C (Brown, Walter, Natan, 2000, Chem Mater, 12, 306-313). Fifty microlitters of 0.1M ascorbic acid, 9mL growth solution (0.25mMHAuCl4, 0.08M cetyltrimethylamonium bromide), and 1.0mL Seed colloid (8.0±0.8nm in diameter) was combined in a glass beaker, followed by continuous stirring for 10 to 20 min at room temperature until the solution turned reddish brown (approximately 17 nm) or brown (37 nm) [10].

D. Enzyme-Linked Immuno-Sorbent Assay (ELISA)

Each microwell of the 96-well Corning plate was pre-treated with 100µL 2% Glutaraldehyde for 20 min at room temperature. One hundred and 50 microliters of 15 mM gold nanoparticles were added to the microwells and incubated for 2 hour at room temperature followed by Milli Q water wash for three times and then washed with 0.5% Triton X-100 in PBS for three-times. Blocking for non-specific binding was performed by adding 100uL of 3% BSA and incubated for 60 min at room temperature followed by PBS wash for three times. Binding was performed by adding 100uL properly diluted antiserum into microwells and incubated for 1 hr at room temperature followed by thoroughly washes. HRP-conjugated anti-mouse IgG, ABTS, and H_2O_2 was incorporated in sequence to the wells according to manufacture's protocol and the binding efficiency was monitored by absorbance at 405 nm. The competition ELISA was performed in an ependorf tube by adding gradually concentrated competitor to the anti-5 nm gold nanoparticle anti serum in a total volume of 100 µL, incubated 1 hr at room temperature, and used as antiserum following the previously described ELISA procedure [11, 12].

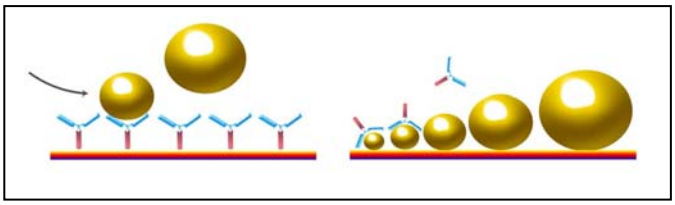

Figure 3. Schematic representation of QCM detection. Antibody-coated electrode detects various sizes of GNPs (left). GNP-coated electrode detects antibody (right).

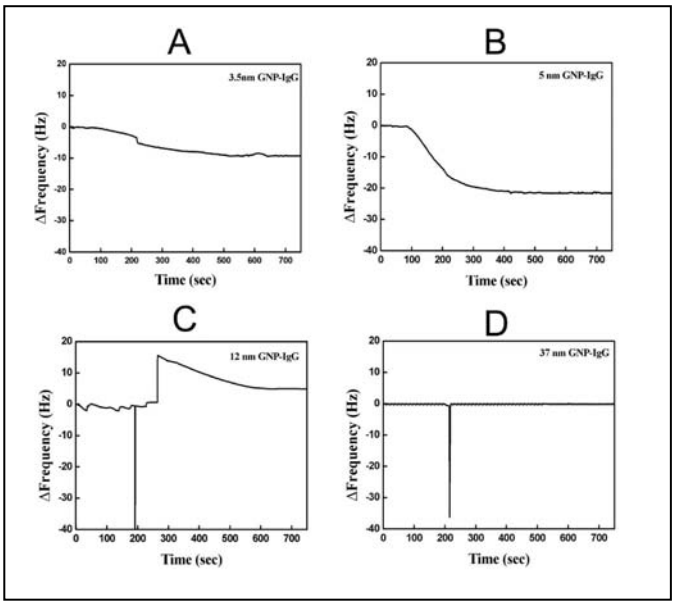

Figure 4. Detection of GNPs using antibody-coated QCM. Frequency shift is monitored for injection of 5 micro-liters of (A) 3.5 nm GNP, (B) 5 nm GNP, (C) 12 nm GNP, and (D) 37 nm GNP.

III. RESULTS AND DISCUSSION

A. Antisera against GNP

We synthesized 5 nm, 8 nm, 12 nm, 17 nm, and 37 nm GNPs according to the published procedure [1]. The synthesis of GNP was monitored by UV absorbance and the size was examined by electron microscopy. The synthesized GNPs were further purified by size-exclusion chromatography and resuspended in phosphate-buffered saline before immunization. GNPs were emulsified thoroughly with complete adjuvant and injected peritoneally and weekly to BALB/C mice. Mice

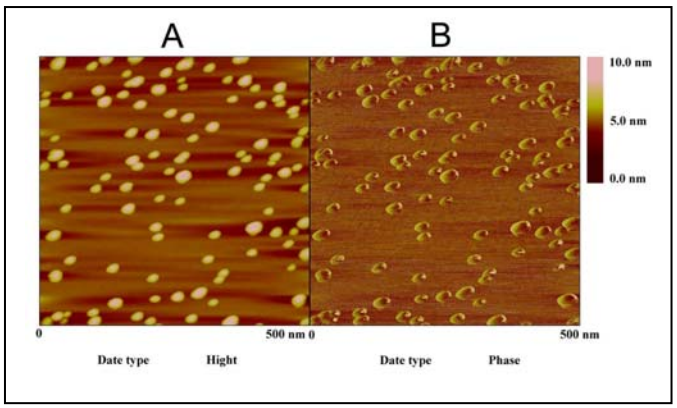

Figure 5. AFM images of 17 nm GNP-coated QCM electrode.

injected with 8 nm, 12 nm, 17 nm, or 37 nm GNP died within 2 weeks, while mice injected with 5 nm GNP were feeble but survived at the end of the fourth week. Antiserum was obtained at the end of fourth week. The binding activity of antiserum against 5 nm GNP was validated by Enzyme-Linked Immunosorbant Assay (ELISA) and a titer of 1000-fold was shown in Fig. 1A. Competition ELISA was performed to validate the binding specificity of antiserum using 5 nm GNP and 5 nm ZnO as competritors (Fig. 1B). The binding activity was blocked in the presence of 5 nm GNP but was undisturbed in the presence of 5 nm ZnO. The antiserum contained specific binding activity to 5 nm GNP but not ZnO.

B. Detection of GNP using antiserum-coated QCM

QCM electrode was coated with antiserum and housed in a QCM-FIA system (Fig. 2). The immobilized immunoglobulin is capable of distinguishing GNPs of different size (Fig. 3). Injection of 3.5 nm and 5 nm GNP induced 5 Hz and 21 Hz frequency shift respectively while other GNPs could not bind to the immunoglobulin-coated QCM electrode (Fig. 4).

C. Construction of flexibility sensor matrix

Detection of immunoglobulin can be achieved by a QCM matrix. Each electrode was coated with single species of GNP of certain size. To construct flexibility-detecting sensor matrix QCM matrix system was assembled as reported (Fig. 2). Six QCMs were housed in flow-cells in an FIA system. Each QCM oscillated independently and controlled by programmable relays. GNPs of 3.5, 5, 12, 17, and 37 nm in diameter were synthesized and coated on each of the gold electrodes of QCM via glutaraldehyde cross-linking respectively. Bovine serum albumin (BSA) was also coated on the electrode and served as blank control. The modified electrodes were examined under AFM to show a typical GNP-coated landscape (Fig. 5). Antiserum was injected into the QCM matrix. Most profound frequency shift occurred to the 5 nm-GNP coated QCM responding to antiserum injection (Fig. 6). QCM coated with 3.5 nm GNP showed moderate frequency shift. The QCM matrix thus served as a "flexibility sensor" for immunoglobulin.

Detection of various flexibility profiles of antisera using the To detect flexibility profiles of antisera obtained from different mice immunized by GNPs ranging from 4 to 6 nm, we increased number of QCM to 10. GNPs coated on the electrode ranging from 1 nm to 12 nm. Injection of antiserum induced a continuous frequency response which peaked at 4, 5, and 6 nm (Fig. 7). Control serum did not induce any frequency shift in such matrix. For different batches of antisera immunized with 5 nm GNP, IgG2 and IgG3 exhibited different amplitudes in the frequency shift profile; however, both profiles peaked at 5 nm indicating that the binding maximized at 5 nm.

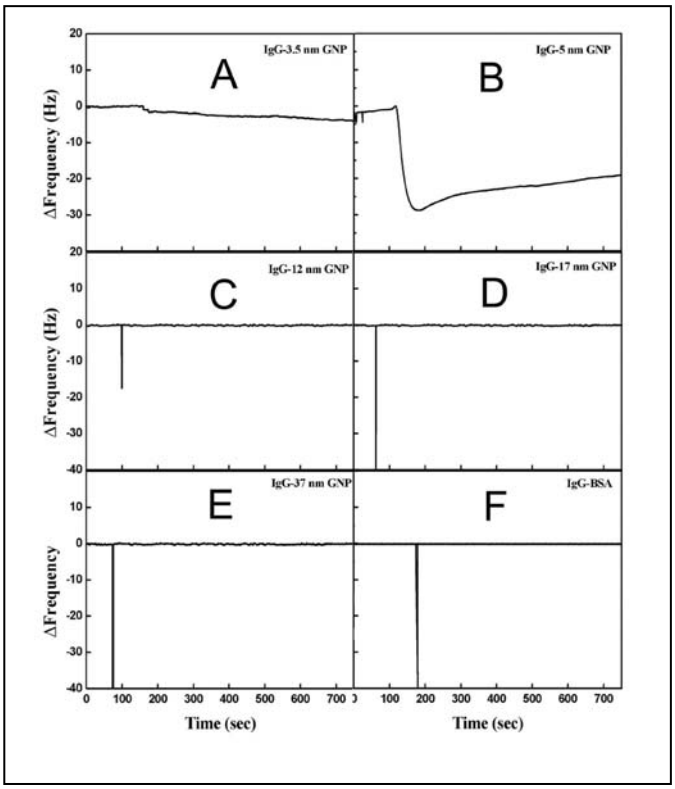

Figure 6. Detection for the flexibility of immunoglobulin using QCM matrix coated with GNPs. Each QCM is coated with 3.5 nm (A), 5 nm (B), 12 nm (C), 17 nm (D), and 37 nm GNP (E). Control electrode is coated with BSA (F).

Detection of biosensor is always specific and the obtained signal is proportional to the amount of detectant bound to sensor. Such sensor detects the "quantity" of detectant. In the current study we constructed a sensor matrix which is capable of detecting the "flexibility profile" of immunoglobulins similar in binding activity but specific to "size" of antigens. The current QCM matrix thus detects both the "quantity" and "quality" of detectants.

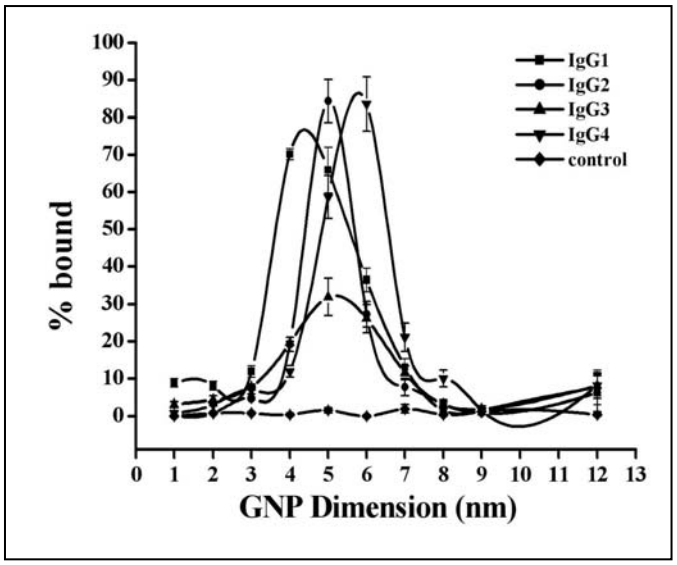

Figure 7. Profiling flexibility of antisera obtained from 4 nm GNP (IgG1), 5 nm GNP (IgG2, IgG3), and 6 nm GNP (IgG4) immunized mice. Control serum was also assayed by QCM matrix.

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ACKNOWLEDGMENTS

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