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Nano-biosensor development for bacterial detection during human kidney infection: Use of glycoconjugate-specific antibody-bound gold NanoWire arrays (GNWA)

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Infectious disease, commonly caused by bacterial pathogens, is now the world's leading cause of premature death and third overall cause behind cardiovascular disease and cancer. Urinary Tract Infection (UTI), caused by *E. coli* bacteria, is a very common bacterial infection, a majority in women (85%) and may result in severe kidney failure if not detected quickly. Among hundreds of strains the bacteria, *E. coli* 0157:H7, is emerging as the most aggressive one because of its capability to produce a toxin causing hemolytic uremic syndrome (HUS) resulting in death, especially in children. In the present study, a project has been undertaken for developing a rapid method for UTI detection in very low bacteria concentration, applying current knowledge of nano-technology. Experiments have been designed for the development of biosensors using nano-fabricated structures coated with elements such as gold that have affinity for biomolecules. A biosensor is a device in which a biological sensing element is either intimately connected to or integrated within a transducer. The basic principle for the detection procedure of the infection is partly based on the enzyme-linked immunosorbent assay system. Anti-*E. coli* antibody-bound Gold Nanowire Arrays (GNWA) prepared on anodized porous alumina template is used for the primary step followed by binding of the bacteria containing specimen. An alkaline phosphatase-conjugated second antibody is then added to the system and the resultant binding determined by both electrochemical and optical measurements. Various kinds of GNWA templates were used in order to determine the one with the best affinity for antibody binding. In addition, an efficient method for enhanced antibody binding has been developed with the covalent immobilization of an organic linker Dithiobissuccinimidylundecanoate (DSU) on the GNWA surface. Studies have also been conducted to optimize the antibody-binding conditions to the linker-attached GNWA surfaces for their ability to detect bacteria in clinical concentrations.
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Abbreviations: **UTI: Urinary Tract Infection; GNW: Gold Nano Wire; GNWA: Gold NanoWire Array; SAM: Self-Assembly of Monolayers; PNPP: *p*-Nitrophenylphenol-Phosphate; LPS: Lipopolysaccharide; DSU: Dithiobissuccinimidylundecanoate; ALPase: Alkaline Phosphatase; ME: β-Mercaptoethanol; EIS: Electrochemical Impedance Spectroscopy; CV: Cyclic Voltametry.**

Introduction

Developing reliable sensing technology for detection of bacterial infection is important from both a commercial and humanitarian standpoint. The rate of infection all over the world has increased for various reasons including emergence of new pathogens. Conventional bacteria detection methods that provide detailed information regarding the nature of infection involve either microscopic observation of the microorganism by tagging fluorescent stains or by culturing a sample in a laboratory for colony growth. Both techniques suffer from relatively poor sensitivity and slow response. Among hundreds of strains

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of *E. coli*, most are harmless, however, a few are quite aggressive for their capability of producing toxins that cause serious illness in humans (UTI, HUS, etc.), sometimes being fatal [1]. The antibiotic treatment for *E. coli* infection is dependent on the detection of the specific strain of bacteria present in the body. Presence of more than 170 strains of *E. coli* have been recognized and their roles in UTI have been implemented to the presence of a specific O antigen which is linked to the core lipopolysaccharide (LPS) of the bacterial cell wall [2]. The specificity of the O antigen is characterized by its carbohydrate composition as well as by the sequence of outer polysaccharide chains. The O-specific polysaccharide moiety of the LPS has been isolated and purified from various virulent strains of *E. coli* and have been confirmed to contain pentasaccharide repeating units joined in the polymer through α1–4 linkage [3]. The O4 polysaccharide has mean molecular mass of 13,800 Da consisting of a core oligosaccharide with about 14 pentasaccharide repeating units. In another study, a mouse model of UTI has been developed where an isogenic mutant deficient in the O4 antigen was shown to be significantly less virulent in all parameters measured [4]. However, K54 (another *E. coli*-specific antigen) knock-outs were as virulent, causing both bladder and renal infections. Thus an important role for O4 antigen moiety of LPS in the pathogenesis of UTI is anticipated. It has been demonstrated that the O-antigen assembly in various strains of *E. coli* is independent of the chemical structure of O-repeat [5]. A general structure of bacterial cell wall LPS is depicted in Figure 1. The *E. coli* cell surface is virtually covered with O-specific chains, which are the principal determinants of the antigenicity and the specific serotype of the bacteria (Figure 1).

The present microbiological diagnostic procedure for detection of *E. coli* infection requires amplification of a single bacterium in a colony, after a time-consuming overnight culture as mentioned previously. However, if a specific strain of infective *E. coli* could be detected faster, effective treatment could start earlier as well. This might lead to possible decline of more serious infectious effects like kidney failure.

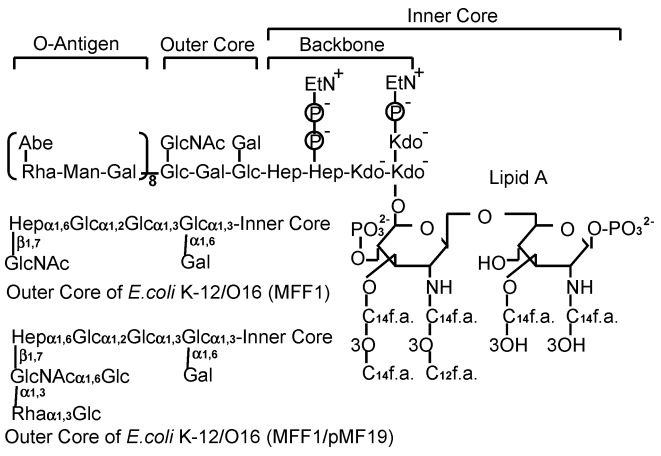


Figure 1. Structure of bacterial lipopolysaccharide.

Currently, major efforts have been directed to the development of a rapid diagnostic detection procedure for *E. coli* infection, in the low physiological concentrations of the bacteria, without amplification. Biosensors have drawn major attention as a diagnostic tool especially in the clinical laboratories and pharmaceutical companies [6,7]. Direct detection biosensors are designed to measure physical changes induced by complex formation between two biological components in a biospecific reaction system, such as antigen-antibody reaction. Depending on the method of assay and the reaction signals, the result of a bio-reaction can be measured either optically or electrochemically, in addition to other methods [7,8]. Clinical analysis has traditionally used reflectance and electrochemical readers that translate reagent response into numerical output [9]. However, increasingly smaller specimen volumes are used requiring lower detection limits as the production of miniaturized devices rise in clinical laboratories. The conventional biosensors generally lack the combination of high-speed detection, highly specific sensitivity, and the ability to be integrated into miniaturized multi-sensor arrays. In recent years therefore, research to develop the technology of enhanced cost-effective portable, selective, and sensitive biosensors capable of producing fast results has intensified [10–12].

The present publication reports on the development of an *E. coli* biosensor, where the recognition element, an anti *E. coli* LPS-specific antibody, is covalently bound to a patterned gold nanowire array (GNWA) posts, as opposed to random arrangement. The thiol mediated binding is much stronger than physical adsorption. The patterned GNWA results in the formation of organized high-density packing of biological elements which increases the area density of active binding sites. The regular orientation of antibodies on GNWA posts, and the organization of posts into patterns contributes to better detection of antigens because of the exposition of more antibody-binding surface areas. Furthermore because of the nano size, GNWA would reduce the electrical double layer effect and enhance mass transfer [13,14].

The goal of the present report is to increase the detection sensitivity of the biosensor to an *E. coli* concentration of 10^3 cells/ml instead of the current detection limit of the conventional *E. coli* sensor, 10^5 cells/ml. Two different kinds of surface structures have been constructed to investigate better orientation of the bound antibody. They are free standing gold nanowires (GNWA) and porous alumina filled with gold nanowires (GNWA-Al_2O_3). Anodized porous alumina template nanowires of various metals are being widely used in nanofabrication field [12,15]. Furthermore, a procedure has also been developed for covalent immobilization of the antibodies to the GNWA surface via an organic linker Dithiobissuccinimidyl-undecanoate (DSU) that greatly enhanced the binding capacity of *E. coli* to both kinds of GNWA surfaces mentioned above [16]. The results in this publication are mainly characterized using an optical measurement for initial studies followed by electrochemical impedance spectroscopy measurement (EIS) method for bacteria [17–20].

Materials and methods

The antibodies against *E. coli*, the alkaline phosphatase conjugated anti-goat 2nd antibody, phosphate buffered saline, *p*-Nitrophenylphosphate, and all other common chemicals used for this study were purchased from Sigma Chemical Company (St. Louis, Mo). The acidic electrolytes like oxalic acid or sulfuric acids as well as other solvents used for electropolishing were purchased from Analytical Reagent. The solution for electroless deposition of gold was Neorum TWB purchased from Uyemera International Corporation. This solution was chosen because of its ability to work at a neutral pH.

Electrode surface fabrication

Gold has been chosen as the support to immobilize biological elements to biosensors for the current project because of its known affinity for biomolecules, especially for its ability to bind to proteins and thiols [21]. Anodization is a common technique for making porous alumina controlling the pore growth by applied current. The anodization process is initiated by annealing the aluminum discs of desired size (0.1 mm × 1.5 cm) to control the grain. The aluminum discs are heated at 1/3rd of its melting temperature for 3 h under a nitrogen atmosphere followed by cooling at room temperature under similar environment. The surfaces of these processed discs are then made smooth by electropolishing followed by anodization in sulfuric acid. The fabrication of a two dimensional pattern of gold circular sections from nanowire (10 nm diameter) embedded in the porous alumina is accomplished in a two-step process, starting with electrodeposition of gold from a $HAuCl_4$-$3H_2O$, H_2SO_4 bath, where gold is seeded inside pores of anodized alumina [12,19,20]. These seeds then become catalytic sites for electroless auto-catalyzed deposition. Electroless deposition in commercial solution from Neorum TWB (Uyemura Corp) is accomplished without electrical power and at a neutral pH. This deposition process allows the control of the length of the gold nano-post inside the pore by controlling deposition time and avoids over-plating gold outside the pores. By this method two different kinds of gold surfaces were generated [20]. In one (Figure 2), the active surface has the gold posts attached to the alumina structure (GNWA-Al_2O_3), while in the other, alumina has been removed from the gold posts by dissolution in NaOH solution for a specific time leaving a gold-toothbrush-like structure (GNWA; Figure 3). A third surface of flat gold, lacking a pattern, was used as a control.

Both procedures are described schematically in Figures 2 and 3. Alumina-backed GNWA (GNWA- Al_2O_3) structures are formed by controlling the time of electroless deposition of gold on anodized porous alumina, so that the gold was deposited just to the top of the porous alumina template surface. Sodium hydroxide solution was then used to dissolve a thin top alumina layer exposing only the very top part of the gold nanowires to bind biomolecules, while the rest of the GNWA is embedded in aluminum oxide (Figure 2). The second type of nanowire surface as described in Figure 3 was made by overdeposition of gold during electroless plating followed by dissolution of the

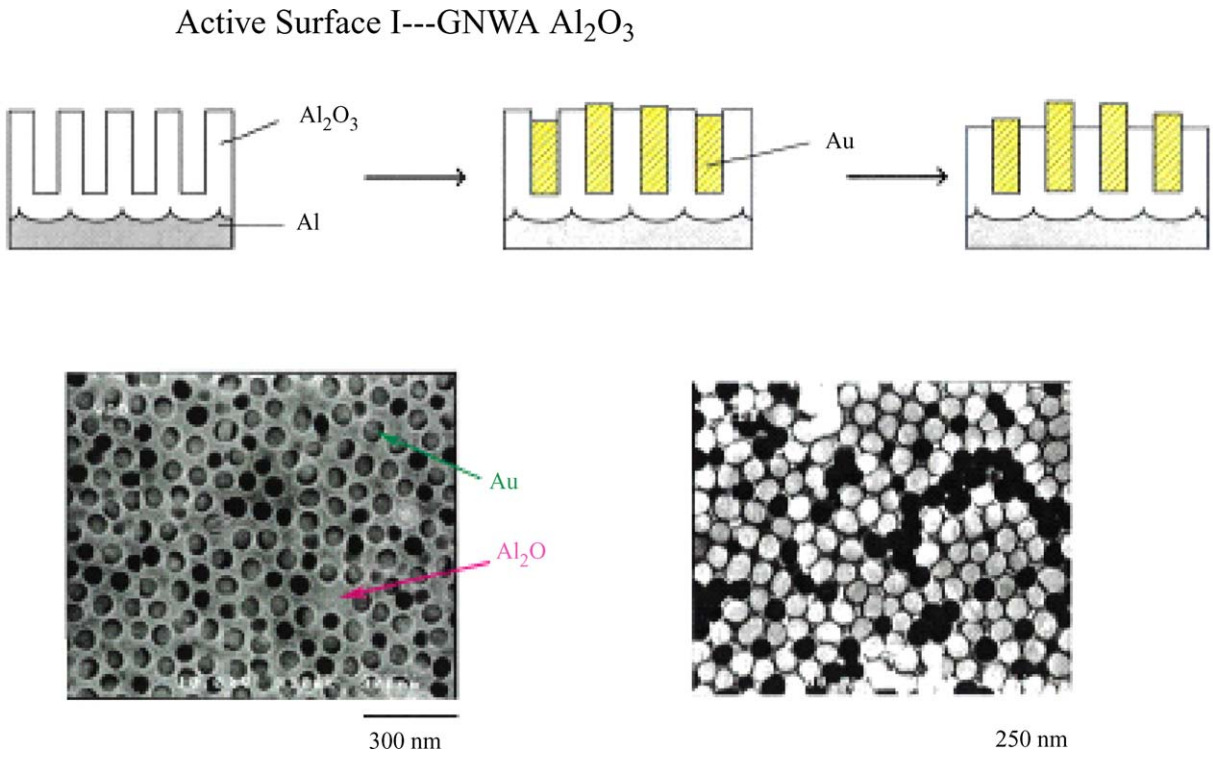


Figure 2. Scanning electron microscopy of Al_2O_3-backed gold Nano Wire (Al_2O_3-GNWA).

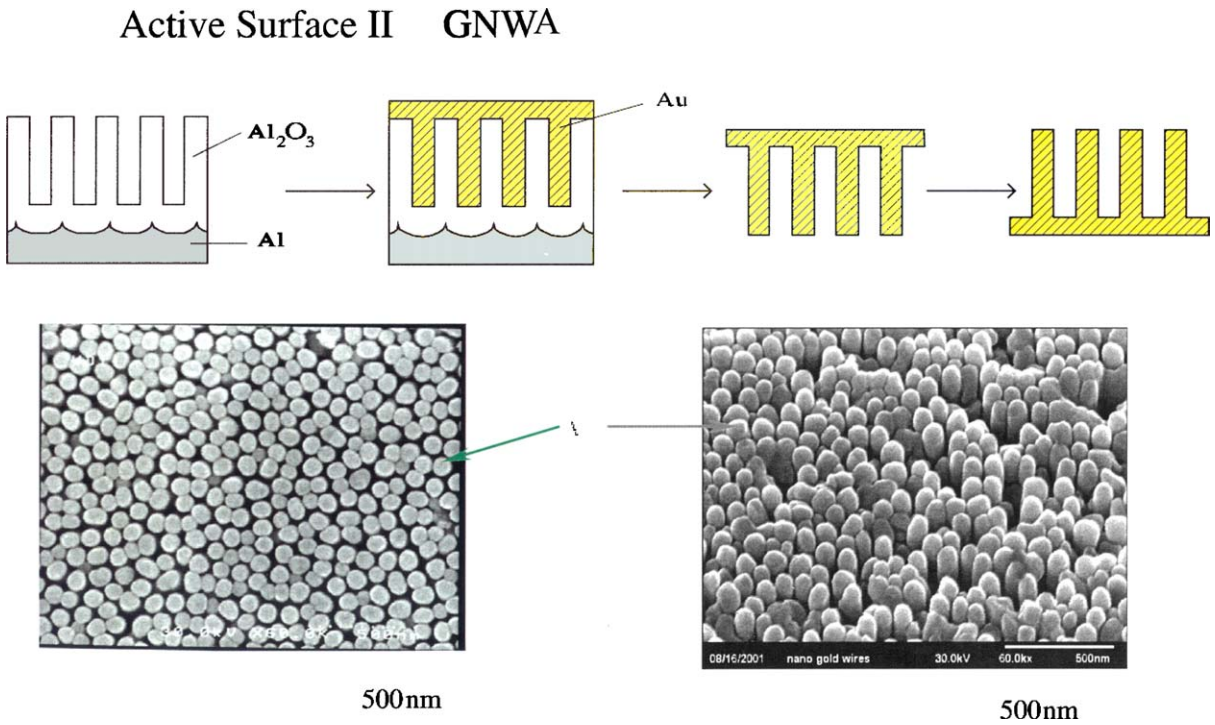


Figure 3. Scanning electron microscopy of gold Nano Wire (GNWA).

aluminum metal and the alumina with 1.5 M NaOH, at 40°C. Toothbrush-like bristles of gold nanowires (GNWA) over a thin gold layer were then protruded from the surface by flipping over the thin gold sheet.

Direct binding of antibodies to gold patterns

The procedure for measurement of *E. coli* binding to different kinds of gold templates followed the basic principle as used for enzyme-linked immunosorbent assay system (ELISA) using an anti-*E. coli* antibody. The ELISA had been performed according to the established method routinely followed in our laboratory as depicted in Figure 4 [22]. Studies were carried out using varying amounts of $(\text{Goat PAb})_{E.coli}$ to determine the relative affinity of the antibody to both the nano-gold templates as described above, without the presence of a linker. Varying amounts of $(\text{Goat PAb})_{E.coli}$ were applied to the gold surface and incubated for 2 h. All discs were washed 3 times in 1 × PBS buffer. To reduce non-specific binding, 1% Casein in 1 × PBS was applied over the gold discs and incubated for 1 h, followed by 3 washings in 1 × PBS. After 30 min, colorimetric assay using a second antibody labeled with ALP was used for the measurement of the first antibody bound to the surface (Figure 4). The second antibody, anti Goat IgG-ALP was applied to the gold discs and incubated for 1 h, followed by 5 washings in 1 × PBS. The antibody-bound gold templates were embedded with 2 ml sodium borate buffer (pH 8.4), followed by 60 μl of 0.01 M p-nitrophenylphosphate (PNPP) and incubated for 30 min. The yellow color, as developed by cleavage of PNPP to

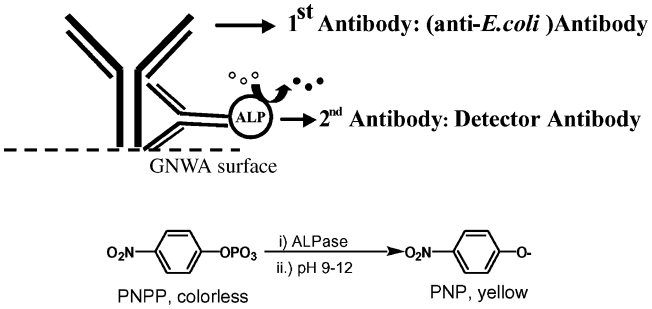


Figure 4. Detection of antibody binding to GNWA surfaces using ELISA protocol.

p-nitrophenol under basic conditions, was estimated by optical density at 405 nm.

Synthesis of dithiobissuccinimidylundecanoate (DSU)

A procedure has also been developed for the covalent immobilization of the antibodies to the GNWA surface via an organic linker that greatly enhances the binding capacity of the *E. coli* antibodies to different kinds of GNWA templates. Dithiobissuccinimidylundecanoate (DSU) was found to be able to immobilize such proteins onto ultra flat gold surfaces [23]. Therefore, DSU was synthesized and prepared for self assembly onto the gold surface templates of both kinds described above [24].

DSU was synthesized according to the following procedure (Figure 5). In the first reaction, 11-bromoundecanoic acid (compound 1, Figure 5) was added to $Na_2S_2O_3$ in 50% aqueous 1,4

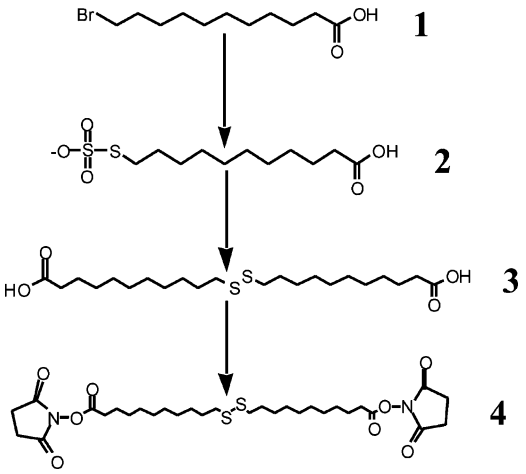


Figure 5. Dithiobissuccinimidylundecanoate (DSU) synthesis scheme.

dioxane and refluxed for 2 h at 90°C to yield the Bunte Salt, (compound 2, Figure 5). The oxidation to the disulfide was carried out by addition of I_2. The I_2 surplus was retitrated with 15% aqueous sodium pyrosulfite. The 1,4 dioxane solvent was removed by rotary evaporation, and the suspension was filtered to yield dithio-bis(undecanoic acid), (compound 3, Figure 5). Purification was achieved by recrystallization from 1:1 Ethyl Acetate/Tetrahydrofuran. *N*-Hydroxysuccinimide was added to a solution of dithio-bis(undecanoic acid) in MeOH, followed by dicyclohexylcarbodiimide (DCC) at 0°C. The reaction mixture was adjusted to 25°C and stirred for 48 h. Methanol was removed by rotary evaporation, and the product was resuspended in Tetrahydrofuran and filtered. The crude final product (compound 4, Figure 5) was purified by recrystallization from 1:1 acetone/hexane, and by Silicic Acid column chromatography to yield DSU, (compound 4, Figure 5). All intermediates were characterized by IR spectroscopy.

Covalent immobilization of antibodies onto gold surface via DSU-SAM

Monolayers of DSU on GNW and GNW-Al_2O_3 templates were formed by immersing the gold structured discs in 1.5 ml DSU solutions (1 mM in MeOH) for 30 min at room temperature. After rinsing with 7 ml MeOH, *N*-hydroxysuccinimidyl (NHS)-terminated monolayers were dried under a stream of nitrogen followed by immediate immobilization of *E. coli* antibodies according to the procedure for direct binding described above. The self-assembled DSU monolayer on gold template as well as antibody-bound DSU-linked GNWA is depicted in Figure 6a and b respectively.

Removal of antibodies from GNW

To determine whether the gold patterns are reusable, a procedure was developed for the removal of the $(\text{Goat PAb})_{E.coli}$ from gold pattern surfaces by washing with 2-Mercaptoethanol, (2-ME). After the completion of ELISA assay, the borate buffer and PNPP were removed from each surface, and each surface was then washed 3 times with 1 × PBS. The samples were then incubated individually with 3 ml of 0.1% 2-ME solution for 1 h after which time 2-ME was discarded from each sample and the samples were incubated with 3 ml of 0.2 M Na_2CO_3 for another hour. All tubes were washed 3 times with 1 × PBS, and ELISA assay was repeated. The thiol group of 2-ME forms self-assembled monolayers on the gold surfaces, thereby replacing the antibodies (Figure 7).

Detection of bacteria by electrochemical impedance spectroscopy (EIS)

EIS was performed on a Gamry workstation (Gamry Instruments), potentiostat FAS-1, in the frequency range from 10^{-3} Hz to 10^5 Hz. An AC voltage of 10 mv (RMS) amplitude was applied to the system around the open circuit potential. All three kinds of gold surfaces (flat control, GNWA, and GNWA-Al_2O_3) with the same area (0.178 cm^2) have been studied for comparison. The antibodies were directly immobilized on each sensor surface without the linker followed by addition of the different dilutions of *E. coli* which serves as antigen, for the EIS measurements. The antigen-antibody complex formation will change the surface properties of the sensor, such as capacitance of the biomembrane, measured by performing EIS in each step of the process. EIS was used to measure the change of this capacitance and then to determine the amount of the GNWA-Ab bound *E. coli* (Figure 8a and b). The measurement of the samples with linker-arm attached surfaces will be performed in future.

Results and discussion

Attachment of antibodies to electrode surface

The pictures of two different kinds of gold surfaces used for this study are shown in Figures 2 and 3, respectively, including the EM-amplified surface area pictures in different magnifications. Procedures for the binding of antibodies to gold surfaces were developed for both free and linker-arm attached templates. To optimize the binding conditions, studies were carried out using varying amounts of different antibodies, with high, medium, and low molecular weight molecules as models (*E. coli*, hemoglobin and FITC). These studies measured the relative affinity of a specific antibody binding to the gold surfaces. After the first antibody binding, the discs were incubated with the alkaline-phosphatase-labeled second antibody, raised against the first antibody. The binding is determined by optical density measurement using PNPP as the indicator as described in the previous section [22]. Electrochemical Impedance Spectroscopy studies were performed also [17–20]. Studies were carried out with different gold surfaces to determine which was most efficient at binding the antibodies.

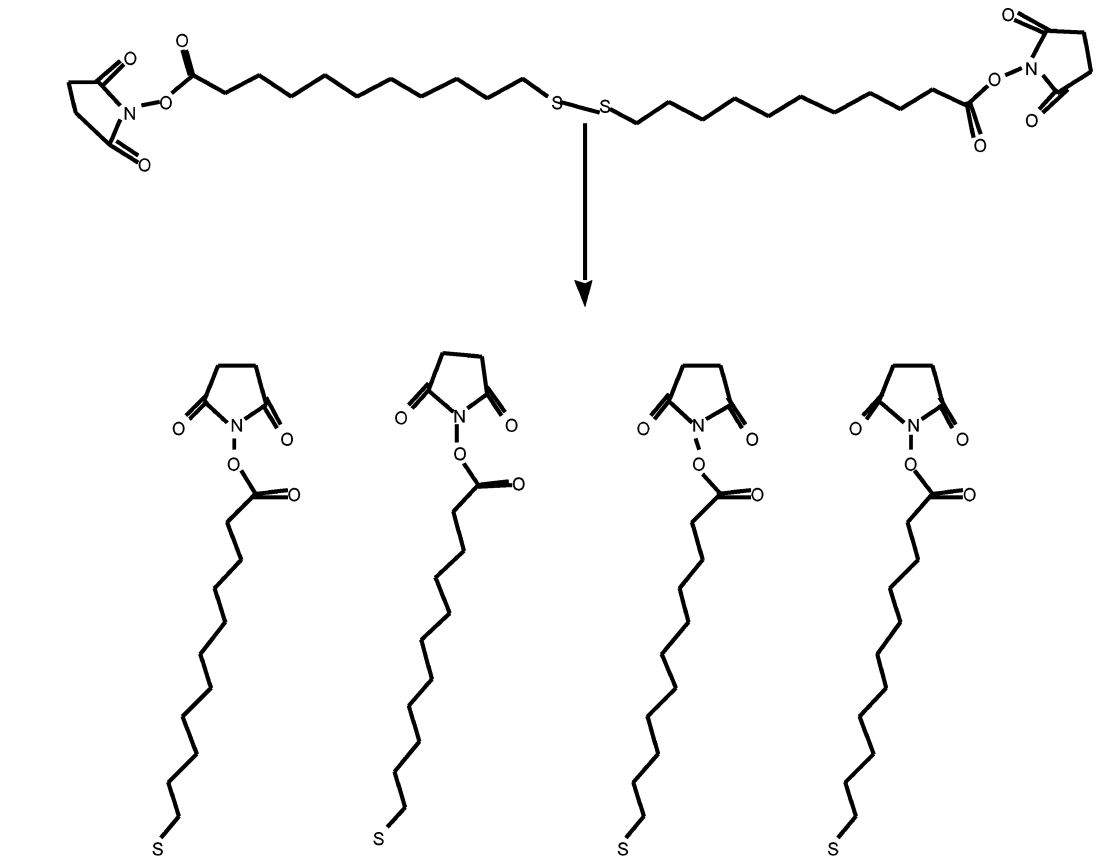


Figure 6. (a) Self-assembly of DSU monolayers on GNW. (b) Covalent immobilization of antibodies via DSU self-assembeled monolayers.

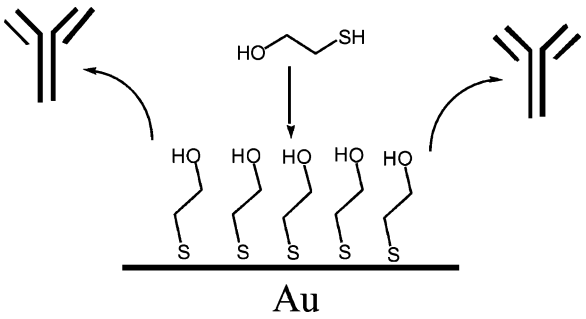


Figure 7. Removal of bound antibody from GNWA surfaces using β-mercaptoethanol.

Binding of Goat $(PAb)_{E.coli}$ to GNW surfaces

Direct binding

Initial studies were carried out using various amounts of (Goat $PAb)_{E.coli}$ to determine the degree of direct binding to both Al_2O_3-backed and free gold surfaces without the presence of any linker (Figures 2 and 3). The results are shown in Tables 1 and 2. The results obtained with flat gold surface used as control are also given in Table 3. Exactly the same amounts of antibodies are used to determine the relative affinities of binding to various surfaces. The results are also given in the same tables when DSU-linked gold templates were used. From the results presented in the tables (Tables 1–3—data without addition of DSU in the assay), the protein binding to all different gold templates seem almost parallel including the control. However, the GNWA-Al_2O_3 surface seems to have the best binding affinity for antibody followed by the free gold wire template (GNWA), while the control flat-gold surface showed the least.

Table 1. Antibody binding (Goat $PAb)_{E.\ coli}$ to Al_2O_3-GNW (direct and DSU-linked discs)

Experiment	*DSU*	*1st antibody*	*2nd antibody*	*O.D. 405*
GNW-Al_2O_3	0	–	6 μg	0.00
GNW-Al_2O_3	0	5 μg	6 μg	0.1924
GNW-Al_2O_3	0	10 μg	12 μg	0.3541
GNW-Al_2O_3	1500 μl	5 μg	6 μg	0.2571
GNW-Al_2O_3	1500 μl	10 μg	12 μg	0.5902

Table 2. Antibody binding (Goat $PAb)_{E.\ coli}$ GNW (direct and DSU-linked discs)

Experiment	*DSU*	*1st antibody*	*2nd antibody*	*O.D. 405*
GNW	0	–	6 μg	0.00
GNW	0	5 μg	6 μg	0.1269
GNW	0	10 μg	12 μg	0.2035
GNW	1500 μl	5 μg	6 μg	0.6481
GNW	1500 μl	10 μg	12 μg	0.6520

Table 3. Antibody binding (Goat $PAb)_{E.\ coli}$ flat gold (direct and DSU-linked discs)

Experiment	*DSU*	*1st antibody*	*2nd antibody*	*O.D. 405*
Flat gold	0	–	6 μg	0.00
Flat gold	0	5 μg	6 μg	0.1442
Flat gold	0	10 μg	12 μg	0.3365
Flat gold	1500 μl	5 μg	6 μg	0.2243
Flat gold	1500 μl	10 μg	12 μg	0.5101

DSU-SAM linked surface binding

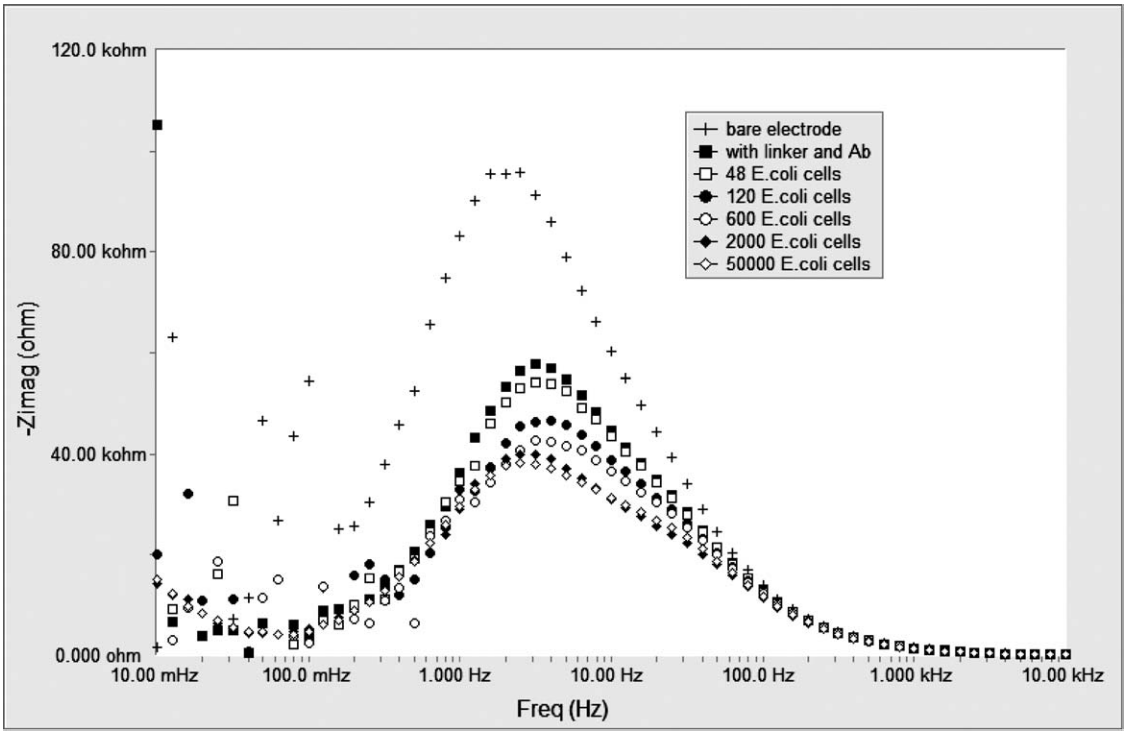
Covalent Immobilization via self-assembly of monolayers is desirable because of the tendency of these monolayers to increase the binding capacity of the gold surface for the antibody. This is due to the fact that the linker has a long carbon chain that protrudes directly up from the plate and can freely rotate in space. This creates a more sterically favorable condition for the antibodies to bind to the monolayers because there is less chance of antibody crowding. Whereas, when antibodies bind directly to the gold surface, crowding may occur on the binding surface decreasing the binding capacity. To determine if antibody binding is enhanced by the presence of the DSU linker, ELISA assays were carried out on all GNW templates with and without the DSU linker as shown in Tables 1–3. The free gold template GNW showed the best affinity for antibody binding when DSU-SAM was present on the surface as shown in Table 2 (data in presence of DSU). The rate of binding is also independent of the protein concentration as seen in the table. Because of the presence of the linker arm, more surface area of the template is exposed for the antibody binding as the value suggested in Table 2 compared to the same in Tables 1 and 3.

Removal of antibodies from GNW

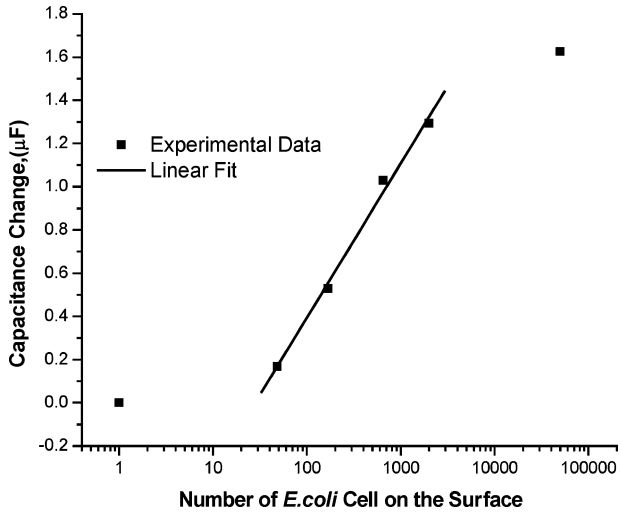
The thiol group of 2-ME forms self-assembled monolayers on the gold surface, thereby removing the antibodies as shown in the Figure 7. Studies were therefore carried out after the 2-mercaptoethanol wash of the antibody-bound gold templates to determine if the $(Goat\ PAb)_{E.coli}$ was rebound to the gold surface. After the completion of ELISA assay, the borate buffer and PNPP was extracted from each surface and discarded. Each surface was washed 3 times in $1 \times$ PBS followed by incubation with ME and washing with Na_2CO_3 as described in the previous section. This creates a basic condition with high levels of OH^- and CO_3^{2-}. Therefore, the gold surface undergoes nucleophilic

Table 4. Antibody binding (Goat $PAb)_{E.\ coli}$ to post-ME washed Al_2O_3-GNW

Experiment	*1st antibody*	*2nd antibody*	*O.D. 405*
GNW	–	6 μg	0.00
GNW	5 μl	6 μg	0.2571
GNW	10 μl	12 μg	0.4686



(a)



(b)

Figure 8. (a) EIS (electrochemical impedance spectroscopy) of Ab-bound GNWA with different amounts of *E. coli* bacteria ($-Z_{imag}$ vs. Frequency). (b) EIS (electrochemical impedance spectroscopy) of Ab-bound GNWA after addition of logarithmic concentrations of *E. coli*. (Capacitance change vs logarithmic *E. coli* concentration).

attack by OH^- as well as CO_3^{2-}. This causes the 2-ME self-assembled monolayers to dissociate from the plate (Figure 7). The results are shown in Table 4 where GNWA-Al_2O_3 was used indicating reusability of the gold templates to fairly good extents after removal of the antibody by washing with β-ME. The same observation was also made when ME-washed free gold template (GNWA) was used.

Electrochemical impedance spectroscopic measurement of antibody binding to gold templates

Impedance spectroscopy is a concept commonly used in electroanalysis. It is closely linked to the concept of resistance, that is the inability of electric current to flow. However, impedance is a more general concept than resistance because it takes into

account the phase difference. The principle is based on applying a small AC potential excitation to an electrochemical cell and measuring the current through the cell. As a result AC current and voltage become out of phase and impedance is then measured as a function of frequency. Impedance magnitude $|z|$, (modulus) can be expressed as an equation with capacitance contributing to the imaginary part [z_{img}] using Euler's relationship. In the current biosensor application, impedance measurements can be used to detect antigen binding to the antibody. This is because the antigen binding to the antibodies linked on the gold surface creates a membrane layer that decreases the conductivity of the surface (increases the capacitance). Therefore, the impedance is increased with antibody binding.

EIS on GNWA surfaces

The impedance is measured for gold surfaces without and with increasing concentrations of covalently linked antibodies as a function of frequency and antigen concentration. The result from the experiments using GNWA surface (no Al_2O_3 backing, Figure 3) is given in Figure 8. Anti-*E. coli* antibody-bound GNWA surfaces were used with various concentrations of antigen, the *E. coli* cells, at concentrations of 0, 48, 168, 648, and 2000 cells per μL (Figure 8a). The gold surfaces are incubated with antigen for 30 min and washed with PBS prior to measurement. The procedure is performed using standard electrochemical impedance system in a Faraday cage and conductance is determined according to the formula below:

$$|Z_{\text{imag}}| = \frac{1}{2\pi f C}$$

where f = frequency in hertz (Hz) and C = capacitance in Faraday (F). If the imaginary part of impedance is plotted against the frequency (10 mHz to 10.00 kHz) different peak-shaped curves were obtained with different numbers of *E. coli* cells (Figure 8a). The peak frequency (3.00 Hz) was used to determine capacitance changes with different numbers of *E. coli* cells (Figure 8b).

From this study, we determined that little if any impedance or capacitance change for the GNWA gold surfaces immobilized with antibody in five separate impedance measurements. Only a 0.04 μF capacitance change was observed for 1000 cells/μL. However, we determined a 30-fold impedance or capacitance change for the free gold surface (GNWA) immobilized with antibody (Figure 8b). This supports the premise that nano-gold patterns are needed for a sensitive EIS detector. A 25 μF capacitance change was observed for 100 cells/μL for GNWA . The detection limit was between 10^{-9} F and 10^{-12} F for the equipment used; therefore, a detection limit of 10 cells over a 0.173 cm^2 area was demonstrated to be possible.

The binding studies with GNWA surfaces above (Figure 8b) indicate that there is a linear relationship between the capacitance and the logarithmic of *E. coli* concentrations in the range of 50 to 1000 cells. However, the sensor will saturate at a higher concentration and thus is much more sensitive in case of free standing nano-wire (GNWA) than the flat gold surface because of the much higher slope of the linear part.

The EIS results with GNWA-Al_2O_3 and Ab-*E. coli* binding studies showed different result than GNWA surface (data not shown) mainly because of the different structure of the sensor. An optimum working frequency range of 0.5 Hz has been determined from the imaginary part of impedance *vs* frequency plot while an increase in capacitance with concentration increase has been observed from capacitance *vs* log *E. coli* concentration plot. This sudden change of impedance might be due to damaged or dead cells. If this is true, our sensor could also be used to detect live cells from the dead cells.

The organic linker, dithio-bissuccinimidylundecanoate (DSU) enhances antibody binding to gold, but not necessarily the EIS signal. The procedure developed for the covalent immobilization of antibodies to gold surfaces via DSU self-assembled monolayers was able to reproduce loading. It was found that the presence of the DSU monolayer increased the binding capacity of the antibodies to gold surfaces

In summary, the present publication reports on a quick detection procedure for bacterial detection during kidney infection based on both optical and electrochemical studies. Detection methods have been developed using a modern technique of nano-sensor as seen with the use of Gold Nano Wire devices in conjunction with a linker arm attached to specific *E. coli* antibodies. The preliminary results indicated that the GNWA-biosensor can detect each of the 50 *E. coli* cells with the sensor area of 0.178 cm^2. A detailed study is necessary; however, this report gives the initial results for this novel procedure to be used in wider applications.

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