Evaluation of Nanomaterials-biomolecule hybrids for signal enhancement of impedimetric biosensors

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Abstract — Five different Nano-materials (NMs) were used for making hybrid conjugates with Protein-A (PrA), a biomolecule that binds specifically with antibodies (Abs). The choice of NMs (Gold, CdS, SrTiO3, Graphene Oxide and Polystyrene) exhibit both inorganic and organic NMs, with a wide range of electrical characteristics i.e., from metal, semiconductor to non conducting dielectric materials. The NMs-PrA conjugates prepared were characterized using UV-visible spectroscopy and fluorescence microscopy before use for biosensor application. These hybrids were then compared for their relative efficacy in enhancing the biosensor signal as recorded using faradaic electrochemical impedance spectroscopy (EIS). A nanogap interdigitated electrodes array (IDEA) sensor was coated with rabbit IgG antibodies and the binding signal from NM-PrA was compared with that of PrA binding alone. The EIS signal was analyzed by fitting the data to a Randles equivalent circuit. Values of two prime circuit elements i.e., charge transfer resistance (R-Ct) and double layer capacitance (C-dl) were determined from the best fit. The difference between the two signals provided the insight into the mechanisms for different NMs and the relative efficiency in signal enhancement.

Index Terms – Nanoparticles, impedance, biosensor

I. INTRODUCTION

Nano-materials (NMs) with attractive physicochemical (electronic, optical, magnetic, thermal, catalytic, etc.) properties have attracted great interest due to their important applications in physics, chemistry, biology, medicine, materials science and interdisciplinary fields. NM-biomolecule hybrid systems, which combine recognition and catalytic properties of biomolecules with these physicochemical properties, are particularly new materials with complimenting properties of both. Nanoparticles (NPs) prepared from different materials that range from metals (Au, Ag) and semiconductors (CdSe, PbS) to dielectric materials (TiO2, SiO2) [1-3]. Nanoparticles offer several advantages that have attracted widespread interest in their use as labels, including simple chemical processes for bio-functionalization, good biocompatibility and size comparable to most biomolecules [4].

The biomolecule-NP hybrid system has excellent prospects for interfacing biological recognition events with electronic signal transduction so as to aid high sensitivity bioelectronic devices. Several different NMs have been used in biosensor application such as inorganic NMs including metal (gold, silver, platinum etc.), semiconducting (quantum-dots) and low dielectrics such as oxides. Organic NMs such as polymers, carbon nanotubes (CNTs), graphene, and bucky-balls have also been reported as materials for enhancing electrochemical biosensors. Even with a large array of NMs available for use with several electrochemical techniques, there is no literature available on comparative performance of these NMs. In this study five different NMs as NPs were conjugated with Protein-A (PrA), a well known 45kda bacterial cell wall protein that specifically binds to antibodies (Abs) [5]. The NP-PrA hybrid exhibits both specific binding and the physicochemical characteristics of the NM such as Gold (Au), Cadmium Sulfide (CS), Strontium titanate (ST), Graphene oxide (GO) and Polystyrene (PS). The latter two nanomaterials being organic, while rest of NMs are inorganic. The electrical properties varied from being highly conductive (Au), to non conducting (PS) through semi-conductive nanomaterial (CS).

Electrochemical transducers have a very important role in healthcare because of the advantage of their technology, which include economy, portability and the possibility of directly identifying and quantifying specific compounds in complex samples. Nanogap interdigitated electrodes were used with EIS as a detection technique as described in our previous work [6]. Abs were immobilized on the transducer electrodes. Both Pr-A and NP-PrA were allowed to bind to the electrode. The EIS data was captured and fitted with an appropriate Randles equivalent electrical circuit (REEC). The difference between the PrA data and NP-PrA hybrid data gave the contribution of the Nanomaterial to the sensor signal enhancement.

II. MATERIAL AND METHODS

A. Chemicals and reagents

Phosphate buffer salined (PBS, 10mM, pH=7.4) and PBS containing 0.05% tween-20 as a detergent (PBST) were used as buffers. Potassium Ferri/Ferrocyanide was used as redox probe couple at an equimolar concentration of 2.5mM for faradic EIS measurements. Protein-A (P6031) and all other chemicals were purchased from Sigma-Aldrich Chemical Company, St. Louis MO (USA) unless otherwise noted. Normal rabbit IgG Abs were a gift from Dr. C.Raman Suri, IMTECH, Chandigarh, India. The interdigitated electrode array chips were fabricated in a SD card format at SHARP labs of America, Camas WA (USA).
B. Nanomaterial-PrA hybrids preparation

Five different NMs in the form of NPs were conjugated to Protein-A. Gold-PrA conjugate was prepared as in [3]. GO-PrA conjugates were prepared in the same manner as for Gold-PrA utilizing the highly negatively charge of GO. ST NPs were conjugated to PrA as in [7]. Briefly, surface amino groups were grafted on ST NPs by treating with an amino silane (APTES). Resulting amino groups were used in carbodiimide based coupling of PrA through activated carboxyl groups. CS NPs coated with amino groups were provided by Dr. Joseph Slocik, AFRL, WPAFB, OH, USA and were conjugated to PrA using the same process as for ST nanoparticles. PS-PrA NPs were obtained from Spherotech, IL, USA and used as such. The prepared conjugates were characterized using UV-visible spectroscopy and Fluorescence microscopy.

C. Biosensor

Interdigitated electrode array (IDEA) was used as electrochemical transducer (fig. 1a). The gold IDEA surface was functionalized using Cysteamine linker displaying free amine groups after formation of self-assembled monolayers (SAMs). Normal rabbit IgG Abs were covalently immobilized on electrodes as in our related work [6].

The impedance measurements were made by scanning the frequency and recording the impedance and phase on a probe station using tungsten-tipped probes and an impedance meter (HP Agilent 4294A). Typically the binding interactions were carried out in PBS containing 0.05% tween-20 as a detergent (PBST) and the electrochemical measurements were made in 10mM PBS containing redox probe (PBSR) consisting of 2.5mM each of K$_4$[Fe(CN)$_6$].3H$_2$O and K$_2$[Fe(CN)$_6$]. The oscillation voltage was kept at 25mV with a DC bias voltage of 220mV. A software application was written in Visual Basic to collect EIS data over a frequency range of 100Hz – 1MHz at the end of the following events: in PBSR before the sample was incubated, after the incubation of the sample (serum dilution), and after the incubation with colloidal gold labels for signal enhancement.

The incubations were carried out in PBST and the buffer was changed to PBSR before data collection. A series of 80 data points were collected per sweep and averaged for three sweeps over a one minute period. The data was analyzed using Z-View software commercially available from Scribner Associates, NC, (USA). The experimental curves were fitted using a suitable Randle's electrical equivalent model. All the plots were made by exporting the analyzed Z-view data to MS-Excel. The signal obtained was used to determine charge transfer resistance (R-Ct) and Double layer Capacitance (C-dl) in presence of PrA and NP-PrA binding. Fig 1b shows the assay scheme used for comparison of different nanomaterial NP-PrA hybrids.

III. RESULTS AND DISCUSSION

A. Nanomaterial-biomolecule hybrid characterization

For all the NMs used in NP form, conjugates prepared with PrA biomolecule were characterized using UV-visible spectroscopy to confirm formation of the NP-PrA hybrid (Fig. 2a).

Fig 2. (a) Shows the UV-visible spectra of Colloidal Au-NP and Au-PrA conjugate. (b) shows a Dot blot assay for confirmation of specific binding of the Au-PrA conjugate with immobilized Ab on membrane and binding of FITC labelled antibodies with ST-PrA nanoparticles forming aggregates.

The biospecific binding of the NP-PrA hybrids was confirmed using a suitable method such as (for gold) a simple dot blot assay that was used with visible confirmation.
provided by the color of the NP binding at the spot of Abs on a membrane (Fig 2b). For CS and ST conjugates formation of fluorescent aggregates in presence of normal Ab and FITC labeled Ab respectively, was confirmed using fluorescence microscopy. Same technique was also used to confirm binding of Fluorescent FITC labeled Abs to PS-PrA and GO-PrA conjugates.

B. IDE as Impedimetric sensor

Faradaic electrochemical Impedance spectroscopy was used to check the sensor performance. All EIS data were recorded in PBSR. Electrochemical impedance sensors are fast emerging as rapid, reliable and cost effective. EIS is a simple but powerful technique for monitoring the changes on the surface of electrodes [8-11]. This technique is advantageous because it can be used to monitor events happening over a broad range of times. Fast events, like electron transfers, can be monitored at high frequencies, whereas lower frequencies can be used to monitor the mass transfer events like those occurring in diffusion of molecules. The interfacial parameters can be easily observed with impedimetric spectroscopy compared to other electrical techniques like chronoamperometry and cyclovoltammetry where current-potential transients are more complex due to double-layer charging and changes in monolayer charge densities during electron transfer. The impedance

![Impedance Plot](image)

Fig 3. (a) Shows the impedimetric spectra of Native IDE electrode with three (R1,R2 and R3) distinct regions. (b) shows the equivalent circuit model used to obtain the best fits (hollow circle curve above) for the experimental data and explain the impedimetric behaviour of the IDEs. Please see ‘IDE as impedimetric sensor’ for details.

phenomena occurring at the interface can be modeled as an equivalent circuit called Randel’s electrical equivalent circuit. The components of the circuit can be related to the interfacial EIS parameters and the experimental data represented with a Nyquist plot.

A typical Nyquist plot obtained using our native IDE device and the equivalent circuit model used for the data fitting and analysis is shown in Fig 3. Three distinct phases (R1, R2 and R3) in the frequency dependent impedance spectra could be determined from the Nyquist plot (Z-real Vs Z-imaginary). First region (R1) arises due to the inherent device resistance and capacitance observed between 1 MHz and 100 KHz, another region (R2) between 5 KHz-100 KHz that arises due to faster charge transfer dynamics at the electrode surface and the region (R3) below 5 KHz was primarily dominated by Warburg impedance (Wo) that arises due to the diffusion dependent mass transfer phenomenon at the electrode surface. The impedance spectra obtained were analyzed by fitting the results in an equivalent circuit model (Fig 3b). Six circuit elements were used for fitting the experimental curve and the model was able to fit the curves in all frequency ranges used in this study. The origin of the plot in the Argand plane was determined by the solution resistance (Rsolv). The shape and diameter of the first semicircular part is determined by the device resistance (R-dev) and Capacitance (C-dev), while values of Charge transfer resistance (R-Ct) and double layer capacitance (C-dl) at the electrode surface determine the second semicircular region and Warburg impedance (Wo) comes into play in low frequency region determining the linear region of the curve.

For the purpose of curve fitting, both C-dl and W-o were implemented using constant phase elements (CPE), both of these components add to impedance characteristics that are attributable to surface roughness of electrodes, chemical inhomogeneity and highly complex ion adsorption behavior at biomolecular layers. Use of CPEs reflects a frequency-dependent resistor in addition to a capacitive element in the equivalent circuit and proved to be flexible and versatile for curve fitting over the whole frequency range as reported in several previous investigations [5,9]. CPE is given by 1/T (jω)^P, where T is analogous to capacitance and ω is angular frequency in rad/s. The CPE (C-dl or W-o) is defined by two values, T and P. When P equals 1 then the equation is identical to that of a capacitor. If P equals 0.5, a 45° line is produced on the complex-plane graph. When a CPE is placed in parallel to a resistor, a depressed semi-circle is produced in the Nyquist plot.

C. Impedimetric Affinity biosensor

A simple model of affinity based biomolecular binding is provided by the well known binding of Protein-A that specifically binds with the Antibodies that are globular proteins produced by mammalian immune system. Protein-A is a bacterial cell wall protein that binds tightly with the IgG antibodies. It was decided to functionalize the immunosensors surface with normal rabbit IgGs and capture Pr-A from the solution. Thus Pr-A when conjugated to the
NM’s would assist the specific binding of NM-PrA hybrids on the sensor surface.

We used cysteamine SAMs for immobilization of biomolecules on the electrodes. The formation of SAMs was confirmed using electrochemical impedance spectroscopy. Both the capacitance and polarization resistance were found to change after SAM formation as compared to that of the native IDEs. R-Ct increased with formation of a subsequent biomolecular layer of capture antibody (Ab) on the electrode surface. The values of R-Sol (143±9 Ω), R-dev (266±57 Ω) and C-dev (3.11±0.41 nF) were found to be relatively unaffected at all stages of the sensor interface and binding experiments. The values of R-ct and C-dl were determined to be 3825±359 Ω and 10.2±3.1 nF for native IDE respectively. These values increased by 15.6, 5.06 and 0.98nF after formation of SAM, Immobilization of normal IgG and capture of Pr-A alone on the electrode surface. The values of R-Ct increased by an average of 929, 448 and 561Ωs at the same stages respectively. The changes in Warburg impedance were not in proportion over the experiment and this R-Ct and C-dl were chosen to be the parameters to compare the signal enhancement due to presence of Nanomaterials at the electrode surface.

D. Biosensor signal enhancement

To compare the effect of different NMs in biosensor signal enhancement, a uniform model of biomolecular binding was required. The binding of PrA with rabbit Abs is highly specific affinity based interaction and quite suitable for our work. So NPs from different NMs were conjugated to PrA and the hybrid was used to bind to rabbit Abs immobilized on sensor surface. The difference in EIS signal due to binding of PrA alone as compared to NP-PrA hybrid provided the contribution of individual NM to the biosensor signal enhancement. A suitable Randles EEC (Fig. 3b) was determined by simulation fitting of the circuit with the observed EIS spectrum using Z-view software. The actual signal was determined by fitting the observed EIS spectra using the same. Fig 4 shows the experimental spectrum and best fits for various at stages of binding comparison of PrA and CS-PrA hybrid for signal enhancement. It was observed that both R-Ct and C-dl changed appreciably for all the NMs tested.

IV. CONCLUSION

A interdigitated electrode array was used to evaluate the relative performance of five different nanomaterials when hybridized with a biomolecular reagent. All the NMs tested showed the capability to influence the observed impedimetric signal in terms of R-Ctand C-dl. Small sized Cadmium Sulfide NPs were found to lead the effects on both of these impedimetric parameters as compared to other nanomaterials tested that included graphene oxide, Polystyrene, Strontium titanate and colloidal Gold nanoparticles for comparison. The results are further being analyzed in our lab to understand the mechanism through which each NM is effecting the biosensor binding signal.

REFERENCES