

4-Fluoro-3-nitrophenyl grafted gold electrode based platform for label free electrochemical detection of interleukin-2 protein



Sunil K. Arya*, Mi Kyoung Park

Bioelectronics Programme, Institute of Microelectronics, A*STAR (Agency for Science, Technology and Research), 11 Science Park Road, Singapore Science Park II, Singapore 117685, Singapore

ARTICLE INFO

Article history:

Received 20 February 2014

Received in revised form

6 May 2014

Accepted 13 May 2014

Available online 22 May 2014

Keywords:

4-fluoro-3-nitrobenzene diazonium ion

Grafting

Interleukin 2 protein

Impedance

Cytokine

ABSTRACT

A new platform based on 4-Fluoro-3-nitrophenyl (FNP) grafted gold disk electrode prepared via electrochemical reduction of 4-fluoro-3-nitrobenzene diazonium ion has been developed and utilized for biosensor fabrication. Anti-interleukin-2 (anti-IL2) antibody has been covalently immobilized onto FNP/Au surface and utilized for label free electrochemical impedance based detection of cytokine IL2. FNP acts as a bridge (cross-linker) between gold surface and anti-IL2, where fluoro group of FNP undergoes nucleophilic substitution by amino group of biomolecule and results in its covalent immobilization. The immobilization process and fabricated electrode have been characterized using contact angle (CA) measurements, cyclic voltammetry (CV) and electrochemical impedance (EIS) technique. CV studies show that FNP grafted surface provides conductive surface for anti-IL2 immobilization. The EIS response of studies as a function of IL2 concentrations exhibits a detection in linear range from 1 pg ml^{-1} to 10 ng ml^{-1} with minimum detectable concentration of 1 pg ml^{-1} . The electrode has been found to be selective against other cytokine molecules.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

In the last decade the ever increasing need for point-of-care biosensors for fast, real-time and reliable medical diagnosis has led to considerable interest in the development of new methods for biomolecular binding and systems capable of the sensitive and specific detection of biomolecules (Arya et al., 2012, 2009; Gallegos et al., 2013; Hecht et al., 2013; Singh et al., 2013; Zhang et al., 2013). Among the various biosensors available today, electrochemical biosensors continue to be popular for their simplicity and capability of direct transduction of the biomolecular recognition event into an electronic signal (Liu et al., 2013; Luo and Davis, 2013). Electrochemical biosensors have shown potential for fast, accurate and sensitive detection of various biologically important analytes (Chen et al., 2013; Huang et al., 2013; Ronkainen et al., 2010; Yang and Zhang, 2010). However, a continuing challenge in the wider use of biosensors, especially in practical settings, has been the sensitivity and stability of the surface bound biosensing molecules. Immobilization of biological molecule is a crucial step in biosensor fabrication and is directly related to the biosensor performance (Corgier et al., 2007; Solanki et al., 2007). Thus, there is a need to develop immobilization process to provide strong, stable and accessible binding of sensing element.

Proper surface modification of conducting or semiconducting substrate for biomolecule binding can provide superior product in terms of biocompatibility, optical/electrochemical properties, electrocatalysis, sensing, etc. (Le Floch et al., 2009). Among various binding techniques, one of the best functionalization routes is through covalent coupling, which ensures a stable and strong binding of desired biomolecule to a substrate (Le Floch et al., 2009). In biosensor fabrication, gold as substrate has proven to be popular surface to work with. Gold provides a conducting platform, which is stable in environment, easy toward chemical modification and easy to characterize. In various biosensors, gold has been used with thiols for coupling of biomolecules in near vicinity of surface (Arya et al., 2009). Thiols spontaneously bind to gold surface and form self-assembled monolayer (SAM) with functional group exposed on surface for subsequent biomolecule binding. The self-assembled monolayers are good candidates for immobilization matrices, since SAMs are easy to form and the properties of SAMs can be easily manipulated via changing functional groups that are compatible for the immobilization of biomolecules (Arya et al., 2013). However, the use of thiol SAM on gold surface has some limitation. SAMs are usually unstable to prolonged contact with air or solution resulting in desorption of SAMs and creation of defects. (Civit et al., 2010; Shewchuk and McDermott, 2009) Also, they can be used in limited potential range and mainly compatible with gold substrate.

Recently, an attractive alternative to thiols has emerged in form of reduction based diazonium ion grafting on various materials of

* Corresponding author. Tel.: +65 90702464; fax: +65 64640517.

E-mail address: sunilarya333@gmail.com (S.K. Arya).

choice (gold, graphite, carbon nanotubes, indium–tin–oxide, iron, etc.) in biosensor (Evrard et al., 2008; Liu and Gooding, 2006; Liu et al., 2007; Mahouche-Chergui et al., 2011; Wang and Carlisle, 2006). Grafting of diazonium ion onto gold results in carbon–gold bond formation, which is more stable than thiol–gold bond. This higher bond strength translates into higher stability of modified surface (Civit et al., 2010). Diazonium grafted layers have shown more stability in terms of resistance to sonication, solvents, etc. (Liu et al., 2007; Shewchuk and McDermott, 2009). The diazonium ion based grafted gold surface has been utilized for corrosion protection, molecular imprinting, protein/DNA binding, biosensor, etc. (Baraket et al., 2013; Corgier et al., 2007; Gam-Derouich et al., 2011; Mahouche-Chergui et al., 2011; Pellissier et al., 2008). Flavel et al. described a simple approach to immobilize protein on electrografted aminophenyl film on silicon surface. The surface was modified via a lithographic process and films were found sufficiently stable to withstand the cleaning steps necessary to remove photoresist (Flavel et al., 2010). Liu et al. described the application of 4-carboxyphenyl grafted electrodes for covalent attachment of oligopeptides for selective electrochemical detection of Cu^{2+} , Cd^{2+} and Pb^{2+} . The diazonium salt/peptide modified gold electrodes showed greater stability and were able to detect very low concentration with high sensitivity (Liu et al., 2007). In another study, Harper et al. described the selective immobilization of DNA and antibody probes on electrode array for simultaneous electrochemical detection of DNA and protein on single platform. In their study, they detected breast cancer BRCA1 gene and interleukin12 (IL12) simultaneously (Harper et al., 2007). Thus, diazonium based grafting can be used for selective patterning of diverse biomolecules on a single device.

The present work describe the application of new and novel diazonium molecule (4-fluoro-3-nitro benzene diazonium ion) based gold grafting and direct binding of anti-IL2 under mild conditions and without further modification. Covalent binding of anti-IL2 was achieved via nucleophilic substitution of fluoro group on 4-Fluoro-3-nitrophenyl (FNP) modified gold surface by amino group of biomolecule. Ethanol amine based washing and serum solution based blocking have been utilized for removal of physically bound anti-IL2 molecules and to prevent non-specific adsorption, respectively. The EIS response of studies as a function of IL2 concentrations exhibits a detection in linear range from 1 pg ml^{-1} to 10 ng ml^{-1} with minimum detectable concentration of 1 pg ml^{-1} . FNP grafted gold surface provides a new platform for binding of biomolecules for sensitive electrochemical detection of various biological analytes.

2. Material and methods

2.1. Chemicals and reagents

4-Fluoro-3-nitroaniline, sodium nitrite, potassium hexacyanoferrate(III), potassium hexacyanoferrate(II) trihydrate and ethanol amine (EA) were obtained from Sigma-Aldrich USA. Fetal bovine serum (FBS) was procured from Biological Industries, Israel. Phosphate-buffered saline (PBS) was purchased from Invitrogen.

Purified anti-human IL-2 antibody (anti-IL2) (cat#500301) and antigen (cat#SRP3085) were from Biolegend and Sigma-Aldrich, respectively. All other chemicals were of analytical grade and were used without further purification.

2.2. Disk electrode fabrication

Disk electrodes with diameter of 3.5 mm were fabricated on 200 mm silicon wafer using standard photolithography techniques. Titanium (Ti; $0.1 \text{ }\mu\text{m}$)/gold (Au; $1 \text{ }\mu\text{m}$) film stack was deposited on the oxidized wafers using electron-beam evaporator (Temescal Inc). Ti layer acts as the adhesion promoter for the gold film. The sensing disk electrodes and connecting pad were patterned by deposition of passive layer. The wafer was passivated with barrier layers of SiO_2 ($0.8 \text{ }\mu\text{m}$)/ Si_3N_4 ($0.2 \text{ }\mu\text{m}$ thick) through plasma enhanced chemical vapor deposition (PECVD). PECVD was conducted at $200 \text{ }^\circ\text{C}$ to prevent inter-diffusion of Ti onto the Au surface. The sensing disk electrodes and bond pads were exposed for electrochemical analysis after photolithography and reactive ion etching (RIE) with CF_4 .

2.3. Preparation of 4-fluoro-3-nitrobenzenediazonium and its grafting onto gold

4-fluoro-3-nitrobenzenediazonium (FNBD) was prepared from 4-fluoro-3-nitro-aniline by diazotization reaction using a slightly modified reported method (Arya et al., 2006). NaNO_2 solution (0.4 g ml^{-1}) prepared in water was added drop wise to the clear and ice cooled solution of 0.8 g of 4-fluoro-3-nitroaniline, dissolved in a mixture of 5.5 ml concentrated HCl and 1 ml water. The reaction mixture was continuously stirred for additional 15 min for completion of diazotization and formation of FNBD. $600 \text{ }\mu\text{l}$ of FNBD solution was utilized for electrochemical grafting experiments to make 4-fluoro-3-nitrophenyl (FNP) layer on gold surface. Before grafting, fabricated electrodes were pre-cleaned with acetone, ethanol and with copious amounts of de-ionized water followed by oxygen plasma for 10 min . Grafting was carried out by electrochemical reduction of FNBD in three electrode cells with disk electrode as working and platinum wires as counter and pseudo reference electrode. Various voltages and time were applied for grafting and optimum response was observed when -0.8 V was applied for 70 s for grafting. Thus, all the experiments were carried out on FNP modified chip prepared using these optimized conditions. Fig. 1 shows the schematic for FNBD generation, grafting and anti-IL2 binding.

2.4. Immobilization of anti-IL2 on FNP/Au electrode

Anti-IL2 ($10 \text{ }\mu\text{g ml}^{-1}$) in phosphate buffer (10 mM , $\text{pH } 7.4$) was prepared as a stock solution. From this stock solution, $10 \text{ }\mu\text{l}$ was poured onto FNP/Au chip to cover disk area completely. For covalent immobilization the FNP/Au electrodes with anti-IL2 solution were incubated at $37 \text{ }^\circ\text{C}$ for 1.5 h in humid chamber. Covalent binding took place via nucleophilic substitution, where nucleophilic amino group of anti-IL2 attacked onto FNP molecule

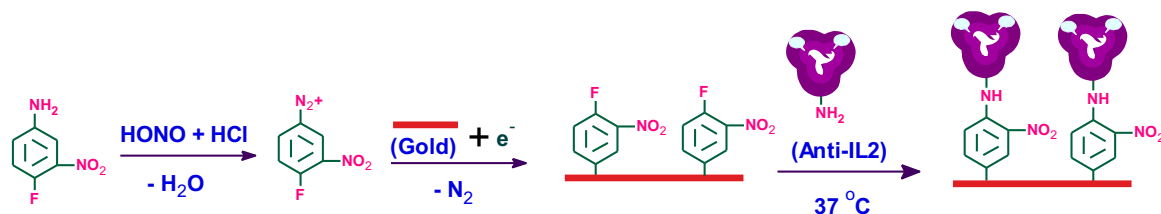


Fig. 1. Schematic for FNBD generation, grafting and anti-IL2 binding.

at 37 °C, to remove fluoro group and made covalent bond with phenyl ring (Fig. 1) (Arya et al., 2006, 2007). The electrode (anti-IL2/FNP/Au) thus, formed was washed thoroughly with phosphate buffer (10 mM, pH 7.4) and 10% ethanolamine solution (10 min) to remove any unbound antibodies. 10% FBS incubation for 1 h was utilized for blocking of non-specific binding. The electrodes were stored at 4 °C when not in use. The fabricated anti-IL2/FNP/Au electrodes were characterized using the contact angle, cyclic voltammetric (CV) and electrochemical impedance spectroscopic (EIS) techniques.

2.5. Contact angle measurements

Contact angle measurements were conducted to check the hydrophilic/hydrophobic nature of the surface before and after modification and the immobilization of antibody by the Sessile drop method using a drop shape analyzer (DSA 100) from Kruss GmbH Hamburg.

2.6. CV and EIS studies

CV and EIS studies were carried out on Autolab Potentiostat/Galvanostat (Eco Chemie, Netherlands) using a three-electrode configuration with disk electrode as working and platinum wires as a pseudo reference and counter electrode in 600 μ l of phosphate buffer saline (PBS) solution ($1 \times$, pH 7.4), containing mixture of 5 mM $[\text{Fe}(\text{CN})_6]^{4-}$ (ferrocyanide) and 5 mM of $[\text{Fe}(\text{CN})_6]^{3-}$ (ferricyanide), i.e. 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ as a redox probe. CV data recording, measurements were carried out in -0.5 to 0.5 V at a scan rate of 50 mV s^{-1} . EIS studies were carried out at equilibrium potential without external biasing in the frequency range of 0.5 – $50,000$ Hz with 25 mV amplitude.

3. Results and discussion

3.1. Contact angle studies

To investigate FNP layer formation and anti-IL2 immobilization, the contact angle measurements were carried out using the Sessile drop method. The change in the value of the contact angle revealed the hydrophobic/hydrophilic character of the surface, which in turn has been related to FNP layer formation by grafting and anti-IL2 immobilization on the gold surface. Fig. 2 shows the contact angle images for bare Au disk electrode, FNP/Au and EA/anti-IL2/FNP/Au electrodes.

It can be seen that the value of the contact angle increases dramatically from 21° for bare Au to 77° for FNP/Au, indicating that the FNP molecules get grafted on the Au surface. The decrease in the value of the contact angle was observed from 77° to 42° after reaction with anti-IL2. The observed decrease in value of the contact angle suggests the hydrophilic nature of the surface

confirming the immobilization of anti-IL2 onto the FNP/Au surface. Measurements were repeated in triplicate on different chips and results were found within $\pm 3^\circ$.

3.2. Electrochemical characterization

3.2.1. EIS studies

EIS is a very sensitive technique and has been used as a characterization tool for studying the charge transfer processes occurring at the sensor-sample interface. Fig. 3(a) shows the step wise EIS based characterization for EA/anti-IL2/FNP/Au electrode fabrication and FBS blocking. Nyquist plots of impedance spectra were recorded after each step of the modification processes and diameter of semicircle was measured to estimate the charge transfer resistance (R_{ct}), which revealed the electron transfer kinetics at the electrode–electrolyte interface. In the present study, Nyquist plots of impedance spectra were found to be the same for repeated scans of a particular surface, and found to change only after each step of modification.

In Fig. 3(a), increase in R_{ct} from 62Ω for bare Au to 952Ω for FNP layer formation was observed, which revealed the dielectric shielding of electrode array by FNP layer formation by electrochemical reduction based FNBD grafting. Increasing R_{ct} indicated that FNP layer formation retards the charge-transfer process. Covalent binding of anti-IL2 (curve (iii)) onto the FNP layer resulted in increase of R_{ct} to 4324Ω due to non-conducting nature of anti-IL2. After the treatment with EA, decrease in R_{ct} to 2964Ω (curve (iv)) was observed. The decrease in R_{ct} can be attributed to the removal of unbound physically adsorb anti-IL2 molecules. Further, in curve (v), the increase in R_{ct} to 8881Ω after serum incubation indicated the blocking of free spaces on the electrode with serum protein molecules. Different anti-IL2 modified electrodes prepared in the same batch were found to exhibit similar EIS response value with maximum variation of $\pm 5\%$,

3.2.2. CV studies

CV has been used to characterize FBS/EA/anti-IL2/FNP/Au electrode fabrication and to investigate the electro-active behavior of the FNP/Au electrode. In the present study, FNP/Au based electrodes were found to be stable during repeated scans and different FNP/Au electrodes prepared in the same batch were found to exhibit similar current value with maximum variation of $\pm 3\%$. Fig. 3(b) shows the cyclic voltammogram of (i) blank gold, (ii) FNP/Au, (iii) anti-IL2/FNP/Au, (iv) EA/anti-IL2/FNP/Au and (v) FBS/EA/anti-IL2/FNP/Au electrodes in PBS buffer (10 mM, pH 7.4) containing 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in the range of -0.5 to 0.5 V. The oxidation current (Fig. 3(b)) of the FNP layer on gold disk (curve (ii), $I = 110.3 \mu\text{A}$) is much smaller than that of the bare gold electrode (curve (i), $I = 158.7 \mu\text{A}$), indicating the formation of FNP layer by grafting. On modification with anti-IL2, the value of the current further decreases to $36 \mu\text{A}$ and can be attributed to the binding of nonconducting anti-IL2 molecules on surface, which

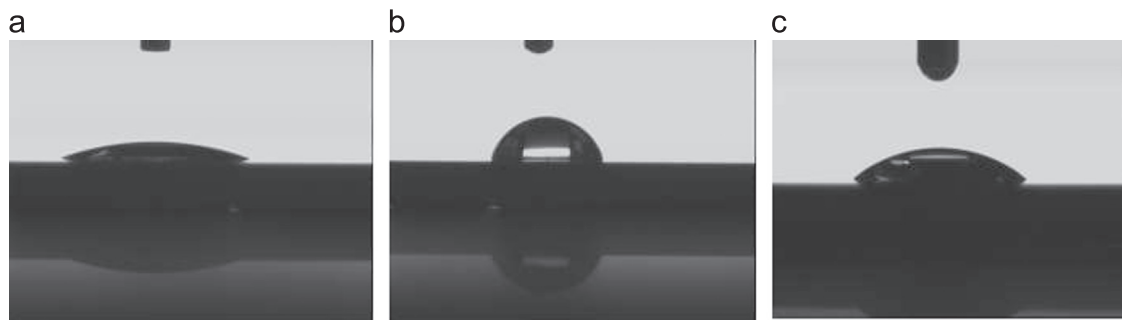


Fig. 2. Contact angle images for (a) bare Au disk electrode, (b) FNP/Au and (c) EA/anti-IL2/FNP/Au electrode.

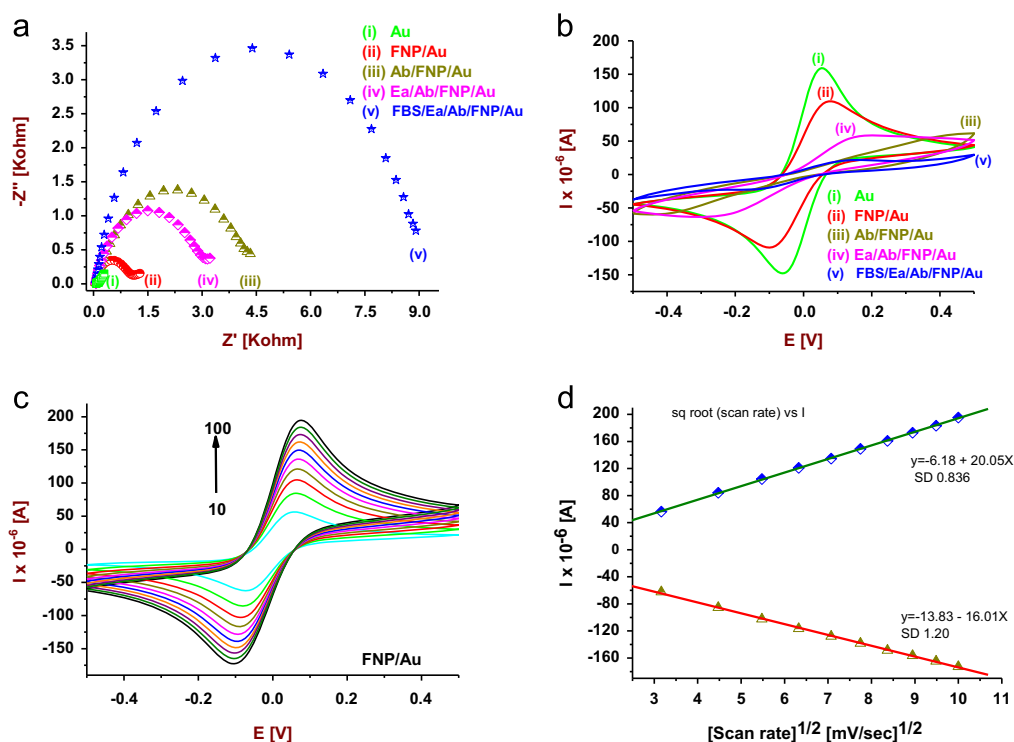


Fig. 3. Characterization of stepwise fabrication immunosensor using (a) EIS (b) CV in PBS (10 mM, pH=7.4) containing 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$. (c) CV studies of FNP/Au electrode as a function of scan rate ($10\text{--}100\text{ mV s}^{-1}$), (d) magnitude of current response vs square root of scan rate.

resulted in decreasing electron flow from solution to electrode surface. After ethanol amine wash, increases in current to $58.5\text{ }\mu\text{A}$ (curve (iv)) was observed and can be attributed to the removal of unbound physically adsorbed anti-IL2 molecules on the FNP modified surface. Finally, after blocking with serum, current was found to decrease to $21.3\text{ }\mu\text{A}$ and is attributed to the serum protein immobilization, which blocks the non-specific adsorption sites and provides hindrance to electron transport.

3.2.3. Scan rate studies for FNP/Au electrode

Fig. 3(c) shows the CV studies of the FNP/Au electrode in a PBS solution (10 mM, pH 7.4) containing 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in the range of -0.5 to 0.5 V at different scan rates (ν), ranging from 10 to 100 mV s^{-1} . It is clear from Fig. 3(d) that the magnitude of current response (anodic (I_{oxi}) and cathodic (I_{red})) for FNP/Au is linearly dependent on the square root of scan rate and follows Eqs 1 and 2. The observed linear relation and well-defined stable redox peaks as a function of scan rate suggests that it is a diffusion-controlled electrochemical process. The consistent peak-to-peak separation and stable peak position/current during repetition at constant scan rate was observed and revealed a quasi-reversible process.

$$I_{\text{oxi}}(\mu\text{A})_{\text{FNP/Au}} = -6.18\text{ }\mu\text{A} + 20.05\text{ }\mu\text{A (s mV}^{-1}) [\nu(\text{s mV}^{-1})]^{-1/2}, \quad \text{with SD } 0.836\text{ }\mu\text{A} \quad (1)$$

$$I_{\text{red}}(\mu\text{A})_{\text{FNP/Au}} = -13.83\text{ }\mu\text{A} - 16.01\text{ }\mu\text{A (s mV}^{-1}) [\nu(\text{s mV}^{-1})]^{-1/2}, \quad \text{with SD } 1.20\text{ }\mu\text{A} \quad (2)$$

3.3. IL2 response studies

Fig. 4(a) shows the EIS spectra obtained on EA/anti-IL2/FNP/Au bio-electrode for IL2 concentrations 1 pg ml^{-1} – 10 ng ml^{-1} . For each concentration, the bio-electrode was incubated in IL2

solution for 20 min, followed by PBS washing and EIS spectra recording using PBS (10 mM, pH 7.4) containing 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ as a redox probe. With increasing IL2 concentration, diameter of the Nyquist plots was found to increase regularly, indicating that IL2 binds to immobilized anti-IL2 on bio-electrode, producing a packed layer that decreases the electron transfer for redox probe. Fig. 4(b) shows a linear relationship between the normalized $R_{\text{ct}}(c_i)/R_{\text{ct}}(c_o)$ values and IL2 concentrations in the range of 1 pg ml^{-1} – 10 ng ml^{-1} . The relationship could be characterized using the following linear equation: $R_{\text{ct}}(c_i)/R_{\text{ct}}(c_o) = 9.14437 + 0.61828 \log C_{\text{IL2}} (\text{g ml}^{-1})$. This biosensing electrode reveals the sensitivity of $0.61828 (\text{g ml}^{-1})^{-1}$ with standard deviation of 0.199. Results of triplicate experiments exhibited similar response within the 5% error for each concentration. Study of IL2 concentration using EA/anti-IL2/FNP/Au electrode revealed that FNP based system prepared via electrochemical grafting may be utilized for highly sensitive biosensor. However, the sensor was found to exhibit high nonspecific binding without blocking. Thus, to make biosensor selective and specific, EA/anti-IL2/FNP/Au electrode surface was incubated with 10% serum solution in PBS for 1 h followed by washing with PBS. The FBS/EA/anti-IL2/FNP/Au electrode was again tested for IL2 concentration.

Fig. 5(a) shows the EIS spectra obtained on FBS/EA/anti-IL2/FNP/Au electrode for IL2 concentrations 1 pg ml^{-1} – 10 ng ml^{-1} . For each concentration, the bio-electrode was incubated in IL2 solution for 20 min, followed by PBS washing and EIS spectra recording using PBS (10 mM, pH 7.4) containing 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ as a redox probe. With increasing IL2 concentration, diameter of the Nyquist plots was found to increase regularly, indicating that IL2 binds to immobilized anti-IL2 on bio-electrode, producing a packed layer that decreases the electron transfer for redox probe. Fig. 5(b) reveals a linear relationship between the normalized $R_{\text{ct}}(c_i)/R_{\text{ct}}(c_o)$ values and the logarithm of IL2 concentrations in the range of 1 pg ml^{-1} – 10 ng ml^{-1} . The relationship could be characterized using the following linear

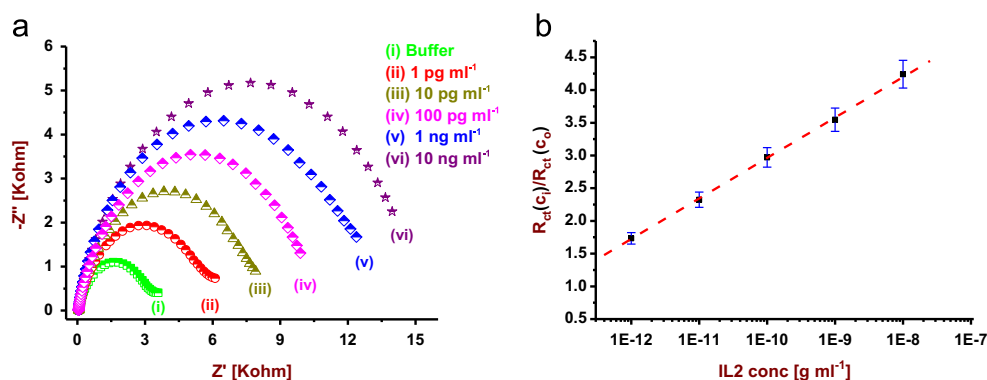


Fig. 4. (a) EIS spectra of EA/anti-IL2/FNP/Au electrode for IL2 concentration (i) buffer, (ii) 1 pg ml^{-1} , (iii) 10 pg ml^{-1} , (iv) 100 pg ml^{-1} , (v) 1 ng ml^{-1} and (vi) 10 ng ml^{-1} . (b) Linearity curve for normalized $R_{ct}(c_i)/R_{ct}(c_o)$ data obtained from EIS studies for different IL2 concentrations.

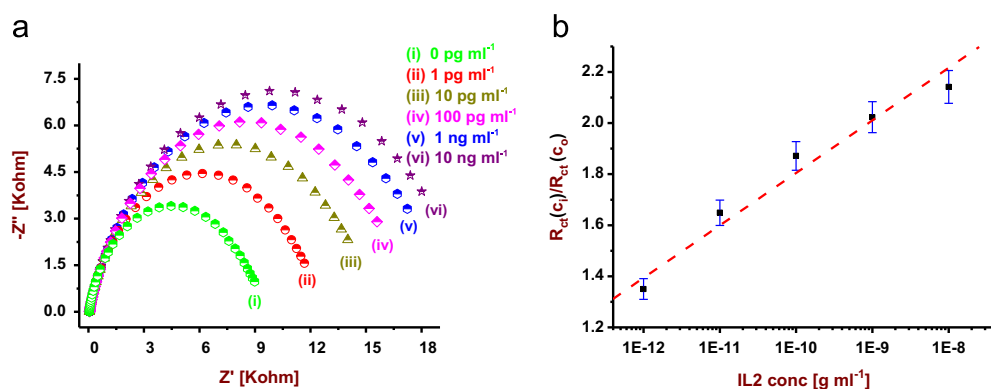


Fig. 5. (a) EIS spectra of FBS/EA/anti-IL2/FNP/Au electrode for IL2 concentration (i) buffer, (ii) 1 pg ml^{-1} , (iii) 10 pg ml^{-1} , (iv) 100 pg ml^{-1} , (v) 1 ng ml^{-1} and (vi) 10 ng ml^{-1} . (b) Linearity curve for normalized $R_{ct}(c_i)/R_{ct}(c_o)$ data obtained from EIS studies for different IL2 concentrations.

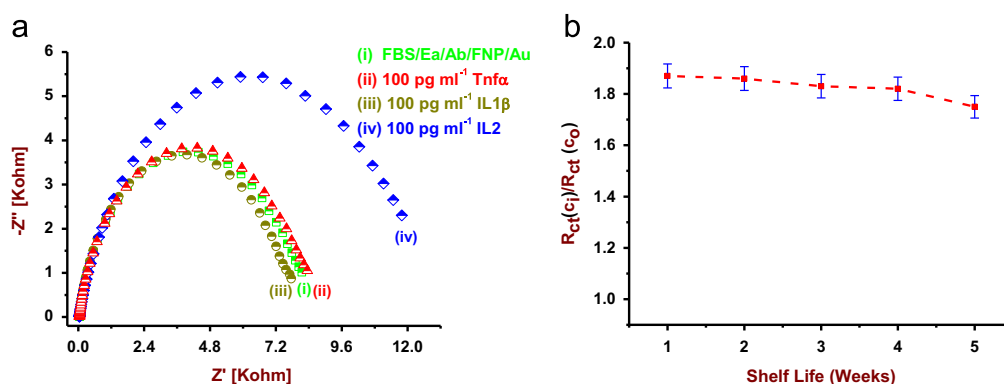


Fig. 6. (a) Nyquist plots of FBS/EA/anti-IL2/FNP/Au electrode in (i) buffer only, (ii) after incubation with 100 pg ml^{-1} of $\text{TNF-}\alpha$, (iii) after incubation with 100 pg ml^{-1} of IL1 β and (iv) after incubation with 100 pg ml^{-1} of IL2. (b) Shelf-life study of FBS/EA/anti-IL2/FNP/Au electrode using 100 pg ml^{-1} concentration.

equation: $R_{ct}(c_i)/R_{ct}(c_o) = 3.86207 + 0.20571 \log C_{IL2} (\text{g ml}^{-1})$. This biosensing electrode reveals the sensitivity of $0.20571 (\text{g ml}^{-1})^{-1}$. Results of triplicate experiments exhibited similar response within the 4% error for each concentration. Sensitivity of FBS blocked surface was found to be lower than without blocking and may be attributed to the reduced nonspecific binding and some hindrance due to the bound FBS proteins. After FBS blocking the electrode was found to be selective and specific for IL2.

3.4. Non-specificity and shelf life studies

The non-specific adsorption was determined by testing the FBS/EA/anti-IL2/FNP/Au electrode against other cytokines such as $\text{TNF-}\alpha$

and IL1 β (Fig. 6(a)). Fig. 6(a) shows the selectivity study results which include the EIS electrode spectra of a buffer only (i), after incubation with 100 pg ml^{-1} of $\text{TNF-}\alpha$ (ii), after incubation with 100 pg ml^{-1} of IL1 β (iii), and after incubation with 100 pg ml^{-1} of IL2 (iv). From Fig. 6(a), it is clear that there was no significant change in R_{ct} with 100 pg ml^{-1} of $\text{TNF-}\alpha$ or IL1 β incubation; however, large change was observed only when electrode was incubated with IL2 solution. Thus, FNP grafted Au surface provides a new platform for biomolecule immobilization near electrode surface and FBS blocking makes the EA/anti-IL2/FNP/Au electrode selective, which can be used for selective estimation of IL2. The shelf life of the FBS/EA/anti-IL2/FNP/Au electrode has been estimated for 1 month using EIS studies at an interval of 1 week.

After 1 month, magnitude of the EIS response was still found to be more than 93%, thus indicating good stability of fabricated electrode, when stored at 4 °C (Fig. 6(b)).

4. Conclusions

In summary, FNP modified Au, a new platform for gold modification and stable binding of biomolecule for biosensor application, has been described. FNP modified gold electrode was prepared via electrochemical grafting of FNBD and used to fabricate an ultrasensitive impedimetric biosensor for IL2 detection. Covalently immobilized anti-IL2 antibody based FBS/EA/anti-IL2/FNP/Au electrode exhibits linear behavior in the concentration range 1 pg ml^{-1} – 10 ng ml^{-1} , with minimum detectable concentration of 1 pg ml^{-1} . The bio-electrode was found to be selective against TNF α and IL1 β . Thus, FNBD grafting on Au results in covalent bond between gold and FNP molecules, and provides reactive fluoro group on surface for facile immobilization of biomolecules. The FNP based method holds high potential to replace a thiol based method to bind biomolecules and to substantially improve binding and stability of biomolecules on gold surface for biosensor fabrication.

Acknowledgment

This work was supported by the Science and Engineering Research Council of A*STAR (Agency for Science, Technology and Research), Singapore, JCO Grant# 11/03/FG/07/03

References

- Arya, S.K., Saha, S., Ramirez-Vick, J.E., Gupta, V., Bhansali, S., Singh, S.P., 2012. *Anal. Chim. Acta* 737 (0), 1–21.
- Arya, S.K., Solanki, P.R., Datta, M., Malhotra, B.D., 2009. *Biosens. Bioelectron.* 24 (9), 2810–2817.
- Arya, S.K., Solanki, P.R., Singh, R.P., Pandey, M.K., Datta, M., Malhotra, B.D., 2006. *Talanta* 69 (4), 918–926.
- Arya, S.K., Solanki, P.R., Singh, S.P., Kaneto, K., Pandey, M.K., Datta, M., Malhotra, B.D., 2007. *Biosens. Bioelectron.* 22 (11), 2516–2524.
- Arya, S.K., Wang, K.Y., Wong, C.C., Rahman, A.R.A., 2013. *Biosens. Bioelectron.* 41 (0), 446–451.
- Baraket, A., Lee, M., Zine, N., Sigaud, M., Yaakoubi, N., Trivella, M.G., Zabala, M., Bausells, J., Jaffrezic-Renault, N., Errachid, A., 2013. *Sensors Actuators B* (0).
- Chen, Y., Yang, Y., Tu, Y., 2013. *Sensors Actuators B* 183 (0), 150–156.
- Civit, L., Frago, A., O'Sullivan, C.K., 2010. *Electrochem. Commun.* 12 (8), 1045–1048.
- Corgier, B.P., Laurent, A., Perriat, P., Blum, L.J., Marquette, C.A., 2007. *Angew. Chem. Int. Ed.* 46 (22), 4108–4110.
- Evrad, D., Lambert, F., Polcar, C., Balland, V., Limoges, B., 2008. *Chemistry – Eur. J.* 14 (30), 9286–9291.
- Flavel, B.S., Gross, A.J., Garrett, D.J., Nock, V., Downard, A.J., 2010. *ACS Appl. Mater. Interfaces* 2 (4), 1184–1190.
- Gallegos, D., Long, K.D., Yu, H., Clark, P.P., Lin, Y., George, S., Nath, P., Cunningham, B.T., 2013. *Lab Chip* 13, 2124–2132.
- Gam-Derouich, S., Mahouche-Chergui, S., Truong, S., Ben Hassen-Chehimi, D., Chehimi, M.M., 2011. *Polymer* 52 (20), 4463–4470.
- Harper, J.C., Polsky, R., Wheeler, D.R., Dirk, S.M., Brozik, S.M., 2007. *Langmuir* 23 (16), 8285–8287.
- Hecht, A., Commiskey, P., Shah, N., Kopelman, R., 2013. *Biosens. Bioelectron.* 48, 26–32.
- Huang, K.-J., Wang, L., Wang, H.-B., Gan, T., Wu, Y.-Y., Li, J., Liu, Y.-M., 2013. *Talanta* 114, 43–48.
- Le Floch, F., Simonato, J.-P., Bidan, G., 2009. *Electrochim. Acta* 54 (11), 3078–3085.
- Liu, Gooding, J.J., 2006. *Langmuir* 22 (17), 7421–7430.
- Liu, G., Bocking, T., Gooding, J.J., 2007. *J. Electroanal. Chem.* 600 (2), 335–344.
- Liu, N., Chen, X., Ma, Z., 2013. *Biosens. Bioelectron.* 48, 33–38.
- Luo, X., Davis, J.J., 2013. *Chem. Soc. Rev.* 42, 5944–5962.
- Mahouche-Chergui, S., Gam-Derouich, S., Mangeney, C., Chehimi, M.M., 2011. *Chem. Soc. Rev.* 40 (7), 4143–4166.
- Pellissier, M., Barrière, F., Downard, A.J., Leech, D., 2008. *Electrochem. Commun.* 10 (6), 835–838.
- Ronkainen, N.J., Halsall, H.B., Heineman, W.R., 2010. *Chem. Soc. Rev.* 39 (5), 1747–1763.
- Shewchuk, D.M., McDermott, M.T., 2009. *Langmuir* 25 (8), 4556–4563.
- Singh, J., Roychoudhury, A., Srivastava, M., Chaudhary, V., Prasanna, R., Lee, D.W., Lee, S.H., Malhotra, B.D., 2013. *J. Phys. Chem. C* 117 (16), 8491–8502.
- Solanki, P.R., Arya, S.K., Singh, S.P., Pandey, M.K., Malhotra, B.D., 2007. *Sensors Actuators B* 123 (2), 829–839.
- Wang, J., Carlisle, J.A., 2006. *Diam. Relat. Mater.* 15 (2–3), 279–284.
- Yang, K., Zhang, C.-y., 2010. *Anal. Chem.* 82 (22), 9500–9505.
- Zhang, G.-J., Huang, M.J., Ang, J.A.J., Yao, Q., Ning, Y., 2013. *Anal. Chem.* 85 (9), 4392–4397.