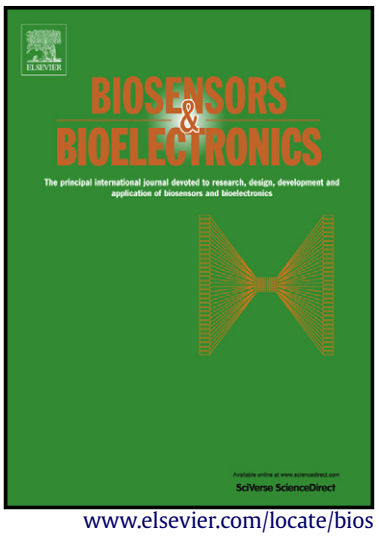

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EIS-based biosensor for ultra-sensitive detection of TNF-α from non-diluted human serum

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Abstract

Serum background is a critical issue for biosensor development as it interferes with the detection of target molecules and may give rise to false positive signal. We present here highly sensitive and selective TNF-α biosensor which is able to detect TNF-α from non-diluted human serum using magnetic bead coupled antibody and electrochemical impedance spectroscopy (EIS) techniques. The process is designed to detect TNF-α from human serum in three stages; (1) abundant protein backgrounds are depleted from the serum using magnetic bead coupled albumin and IgG antibodies, (2) after background depletion TNF-α is captured using magnetic bead coupled TNF-α antibody, and (3) the captured TNF-α is eluted from the magnetic beads and measured using EIS technique in which comb structured gold microelectrodes arrays (CSGM) is utilized to enhance the detection sensitivity. The system is able to achieve the limit of detection

(LOD) at 1 pg/ml (57 fM) and a linear relationship between increasing TNF-α concentrations and charge-transfer resistance at a dynamic range of 1pg/ml – 1000 pg/ml.

Keywords: Tumor necrosis factor, TNF-α, electrochemical impedance spectroscopy, EIS, serum, non-diluted serum

1. Introduction

Tumor necrosis factor (TNF) or TNF-α is a type of cytokines known to induce several signaling cascades leading to subsequence diverse cellular responses such as cell death, survival, differentiation, proliferation and migration (Bradley, 2008; Idriss and Naismith, 2000). TNF-α is also well known for its role as a key regulator in inflammation. Inflammation is a cellular response of innate and adaptive immune system which is triggered by noxious stimuli and conditions such as infections and tissue injuries (Medzhitov, 2008). The role of TNF-α in inflammation is critical in many diseases including rheumatoid arthritis (Feldmann et al., 1996), ankylosing spondylitis (Coates et al., 2010), inflammatory bowel disease (Van Deventer, 1997) and psoriasis (Kyle et al., 2005). Blocking of TNF-α action could improve the diseases prognosis. With its clinical importance, monitoring of plasma TNF-α could help in disease diagnosis and disease progress inspection.

Common laboratory assays employed to detect TNF-α are enzyme-linked immunosorbent assay (ELISA) (Bao et al., 2013; Zhang et al., 2014) and other immunoassays based on principle similar to ELISA e.g. radioimmunoassay (Kenison et al., 1990), time resolve fluorescence assay (Leister et al., 2011). These assays are time consuming processes, involve the use of match-pair antibodies and require several washing steps. Real time detection of TNF-α can be performed

using surface plasma resonance (SPR) technique (Battaglia et al., 2005), in which the change in refractive index of biomolecule bound surface is utilized as a mean of detection (Cottier et al., 2007; Homola et al., 1999). This technique is based on optical method and requires complex equipments that can only be run in central labs.

Numbers of TNF-α biosensor have been developed to simplify the detection procedure, shorten the detection time, improve the detection sensitivity, and reduce the detection cost-per-data point (Cesaro-tadic et al., 2004; Law et al., 2011; Li et al., 2005; Liu et al., 2013, 2012; Pui et al., 2013). Optical detection such as bio-photonic methods gives high quality data therefore has often been employed for the detection of TNF-α (Cesaro-tadic et al., 2004; Law et al., 2011; Li et al., 2005). However, optical method requires complex equipments, thus the development of alternative methods that give high quality data with simple procedure is desired.

Electrochemical immunosensor especially electrochemical impedance spectroscopy (EIS) has recently gained much attention in the field of biosensor developments (Arya et al., 2013; Dong et al., 2013; Hu et al., 2013; Kongsuphol et al., 2014; Li et al., 2010; Pui et al., 2013). EIS is a technique used to study the system response to the application of periodic small amplitude ac signal at different frequencies (Grieshaber et al., 2008; Lasia, 1999). In faradic impedance, information obtained from the system response includes capacitance and interfacial charge-transfer resistance (Grieshaber et al., 2008; Lasia, 1999). These signals can be used to sense different reactions occur on electrode surface. EIS is a very attractive technique for biosensor development as it is a non-destructive method that gives high quality data by directly converting biological event into electrical signal. In addition, EIS system set up, as compare to optical system set up, is small and portable; thus it is possible to perform EIS analysis outside of central lab.

At present, the trend in biosensor development is moving toward the Point-of-Care (POC) application where medical applications are conducted at the patient bed site and the samples are collected on the site (Kumar et al., 2013; Lee, 2008; Vasan et al., 2013), therefore use of blood or serum as a sample is unavoidable. This is a critical issue for biosensor development because blood and serum contain high background content of cells, proteins, electrolytes, lipid, etc (Krebs, 1950) which may interferes with the detection signal and possibly gives rise to the false positive signals. Example of very high serum protein backgrounds are albumin and IgG which accounted for up to 50 mg/ml and 10 mg/ml respectively. Several methods attempting to reduce the serum background interference have been reported (Baker et al., 2006; Du et al., 2010; Lao et al., 2009; Luo et al., 2013; Piliarik et al., 2010; Qi et al., 2013; Rubin et al., 2014), among which the simplest method is to dilute the serum sample before subjecting it to subsequence interventions (Baker et al., 2006; Du et al., 2010; Lao et al., 2009; Piliarik et al., 2010; Rubin et al., 2014). This method is an effective way of reducing the serum background however the target molecule will also get diluted. The diluted serum sample lowers the chance of the target molecule to be interacted with the sensing matrix therefore weak or absence of signal is reflected. Another frequent utilize method to cope with the background interference is the 'blocking' method (Arya et al., 2013; Kar et al., 2012; Liu et al., 2013, 2012; Pui et al., 2013). In principle, the sensing matrix is pre-exposed to the blocking agent in order for the blocking agent to fill up the non-sensing space therefore blocking the nonspecific interaction of the background to the non-sensing area. Although this could prevent background interference, it reduces the sensitivity of the sensing matrix.

We have reported previously the high sensitivity EIS-based TNF-α biosensor which is able to detect TNF-α released from cell culture. Nonetheless, it was observed that the fetal bovine

serum presented within the cell culture media interfered with the TNF-α signal (Pui et al., 2013). Continuing from our previous study in solving the serum background interference, we present here a technique for highly sensitive TNF-α sensor which is able to detect TNF-α from non-diluted human serum using magnetic bead coupled antibody and EIS techniques. In this paper, we present a proof-of-concept of 3-step process for the serum background removal, TNF-α capture, and TNF-α detection, which can be implemented in automated microfluidic system. (1) Serum background removal—the effect of background interference is minimized by removing the abundance serum protein background using magnetic bead coupled albumin and IgG antibodies. (2) TNF-α capture—following the background removal, TNF-α is captured using magnetic bead coupled TNF-α antibody. (3) TNF-α detection—after being captured, the TNF-α tagged beads are separated and TNF-α is eluted from the magnetic beads and measured using EIS technique to record the impedance spectra. To enhance the detection sensitivity, comb structured gold microelectrode array (CSGM) is used as a platform to adsorb TNF-α and record impedance spectra. The system shows the limit of detection (LOD) at 1 pg/ml and a linear relationship between increasing TNF-α concentrations and normalized charge transfer resistance (R_{ct}) at dynamic range of 1-1000 pg/ml. Altogether, this study represents a successful proof-of-concept of a method to detect TNF-α from non-diluted serum which can be further developed into an automated microfluidic system. In addition this study provides a basis for development of biosensor which is able to detect the target molecule from non-diluted serum.

2. Materials and Methods

2.1. Chemicals and reagents

Chemicals and reagents were purchased from the following sources, anti human TNF-α antibody and recombinant human TNF-α from Biolegend (USA); anti human albumin, anti human IgG antibodies, N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), N-Hydroxysuccinimide (NHS), sodium dodecyl sulphate (SDS), and human serum from Sigma Aldrich, (USA); carboxylated magnetic nanoparticles from Sphereotech Inc. (USA); phosphate buffer saline (PBS) from Biowest (USA); other chemicals are with analytical grade from Sigma Aldrich (USA).

2.2.Sample and magnetic-bead coupled antibody preparations

For sample preparation, prior to the experiments, different amount of recombinant human TNF-α were spiked into PBS or pure human serum to obtain final concentrations of 1, 10, 100, and 1000 pg/ml.

For magnetic-bead coupled antibody preparation, 200 µl of carboxylated magnetic beads (0.31 µm, 2.5% w/v) were pre-cleaned with PBS for 3 times followed by incubating with mixture of 0.4 M EDC and 0.1 M NHS in PBS at room temperature for 30 min. The EDC/NHS mixture was removed from the magnetic beads and the beads were incubated with 200 µl of either 250 µg/ml anti human albumin antibody, 250 µg/ml anti human IgG antibody, or 50 µg/ml anti human TNF-α antibody for 1 hr at room temperature. Unbound antibodies were removed from the beads and the beads were resuspended in PBS and store at 4°C until use.

2.3.Electrode fabrication

Comb structured gold microelectrode arrays (CSGM) were fabricated on oxidized silicon wafer using standard photolithography technique as described in an earlier report (Pui et al., 2013). For CSGM electrodes 5 μm wide and 3200 μm long electrode bands with 15 μm spacing, distributed over 5500 μm length were fabricated and utilized. Ships were pre-cleaned by sonicating for 10 min in acetone followed by rinsing with ethanol, copious amount of de-ionized water, and finally cleaning for 20 min under UV-ozone.

2.4.TNF-α capture and EIS measurement

Before capture of TNF-α, abundance background proteins was removed from the serum containing TNF-α sample (50 μl) using magnetic bead coupled albumin and IgG antibodies. Different amount of magnetic beads were tested and best results were observed when 6 μl of anti albumin and anti IgG magnetic beads were used. This condition was employed for the present study in which the sample was incubated with magnetic-bead coupled albumin and IgG antibodies for 1 hr at room temperature. After background removal, the magnetic beads were separated using magnetic rack and the sample was transferred to a fresh new tube for capture of TNF-α. Magnetic bead coupled TNF-α was used to capture TNF-α in which different amount of the magnetic beads were tested and the best results were observed when 3 μl of the magnetic beads were used, thus this condition was applied for the present study. The sample was incubated for 1 hr with magnetic bead coupled TNF-α antibody at room temperature. TNF-α bound magnetic beads were washed with copious amount of PBS and TNF-α was then eluted from the magnetic beads. 250 mM glycine (pH 2) and 2% SDS were tested for TNF-α elution in which elution with SDS was observed to be more efficient than glycine therefore SDS elution was employed for the present study. TNF-α elution was done using 40 μl of 2% SDS in 0.5 M Tris

(pH 7.0) for 10 min at 63°C (Dry bath incubator, Major Science, USA). For TNF-α detection, 40 µl of the eluted sample was incubated on CSGM electrodes for 15 min followed by injection of 40 µl redox probe [5 mM [Fe $(CN)_6]^{4-}$ (Ferrocyanide) and 5 mM of $[Fe(CN)_6]^{3-}$ (Ferricyanide) i.e., 5 mM $[Fe(CN)_6]^{3-/4-}$ in 10 mM PBS solution, pH 7.4] on to the electrode and finally impedance spectra recording and analyzing using EIS technique. EIS measurements were carried out in a three-electrode configuration with gold as the counter and pseudo reference electrodes at the frequency range of 0.5Hz– 500 KHz with a 25 mV amplitude using Reference 600^{TM} (Gamry Instrument, USA). All EIS measurements were performed with consistent parameters at room temperature. Prior to the experiments, blank chips are tested for the initial impedance recordings to verify the baseline R_{ct} (diameter of the Nyquist plots) values which will be further used as reference points. Each experiment was conducted at least in triplicate.

3. Results and Discussions

3.1. Detection of TNF-α spiked in buffer solution

Fig. 1 represents the scheme of the overall system design for detection of TNF-α from non-diluted serum. To reduce to the effect of background interference, albumin and IgG, the serum most abundant proteins, were removed from the serum sample by incubating the serum containing TNF-α with magnetic bead coupled albumin and IgG antibodies (Fig 1a). The albumin and IgG removed sample was transferred to a new tube and TNF-α was then captured by incubating the sample with magnetic-bead coupled TNF-α antibody (Fig 1b). After being captured, TNF-α was eluted from the magnetic beads by incubating with 2% SDS at 63°C (Fig 1c and d). The eluted sample was then underwent impedance spectra recording using EIS technique in which the CSGM electrode was employed for EIS measurement to enhance the detection sensitivity (Fig 1e).

To ensure that the proposed system is feasible, several sets of optical measurement were performed (supp. Fig. S1 and S2). Data obtained from optical measurements suggest that TNF-α could be captured and eluted from magnetic beads (Fig. S1) and serum proteins could be, in a large extent, depleted using magnetic beads coupled with albumin and IgG antibodies (Fig. S2). The system was further tested to detect TNF-α spiked in a non complex solution, in this case PBS. Fig. 2a represents Nyquist plots of eluted samples from 1-1,000 pg/ml TNF-α spiked in PBS. The plots show increase in R_{ct} (diameter of the semicircle) values upon increasing TNF-α concentrations. R_{ct} values obtained from TNF-α are normalized with R_{ct} from blank chips ($R_{ct(norm)}=R_{ct(TNF\alpha)}/R_{ct(blank)}$) and plotted against different TNF-α concentrations. Fig. 2b represents a linear relationship between increasing TNF-α concentrations ranging from 1-1,000 pg/ml and normalized R_{ct} values with coefficient of determination (R^2) value equal to 0.91. These results suggest that the proposed sensor system is feasible and able to detect TNF-α spiked in PBS solution, in which an increase in TNF-α concentrations causes increasing amount of TNF-α adsorption thus obstructing the interfacial charge transfer process therefore increasing the R_{ct} values.

3.2. Detection of TNF-α from non-diluted human serum

Next, the ability of the sensor system in detecting TNF-α from non-diluted serum was determined. Fig. 3 represents Nyquist plots (a) and normalized R_{ct} values (b) of different TNF-α concentrations spiked in human serum. The Nyquist plots show increase in R_{ct} values upon increasing TNF-α concentrations (Fig. 3a), and the normalized R_{ct} values shows proportional linear relation to an increase in TNF-α concentrations of up to 1000 pg/ml with LOD value at 1 pg/ml (Fig. 3b). Data obtained from serum samples (Fig. 3) are comparable with PBS samples

(Fig. 2), thus suggesting that the sensor is capable of detecting TNF-α from non-diluted human serum with the same efficiency achieved in non complex solution i.e. PBS, in which the effect of background interference did not affect the detection sensitivity.

It has been reported previously that TNF-α released from cell culture can be detected (Pui et al., 2013). In that study, the inverse relationship between TNF-α and normalized R_{ct} values was observed and was attributed to the interference effect of serum background present within the cell culture media. The mechanism was explained as followed: At low TNF-α concentrations, TNF-α in combination with the non-specific large size and abundant biomolecules are bound to the biosensor matrix, obstructing charge transfer process thus elevating R_{ct} values, whereas at high TNF-α concentrations, TNF-α are specifically and preferably bound to the biosensor matrix, due to its small size (17 kDa), promoting charge transfer process thus lowering R_{ct} values (Pui et al., 2013). The results obtained from the present work, however, oppose these findings in which proportional relationship between increasing TNF-α concentrations and R_{ct} values has been observed. This is largely due to the background depletion process which plays a crucial role in lowering the effect of serum background interference thus recovering the specific detection of TNF-α signal. Altogether, the results obtained from the present study represent an improved TNF-α biosensor platform which can be used to detect TNF-α from non-diluted serum.

Comparison of the present TNF-α sensor and other electrochemical sensors is demonstrated in table 1. For most of the sensors, PBS or culture media without serum are utilized as diluents thus the effect of serum background interference are not being encountered. Liu et al. (2013) have demonstrated electrochemical-based apta-sensor for detection of TNF-α from blood sample however the effect of background interference has not been discussed. In their study, the

LOD was reported to be 10 ng/ml which is 10,000 time higher than the present study. TNF-α ELISA assay has been conducted as a benchmarking study in which the result obtained from ELISA shows LOD value of approximately 15 pg/ml and dynamic detection range at 15-1000 pg/ml (Supp. Fig. S3). The TNF-α sensitivity obtained from ELISA is 15 times less sensitive as compare to the present biosensor.

3.3. Selectivity study of TNF-α biosensor

To determine the system specificity to TNF-α, serum containing interleukin-2 (IL-2) were used as control samples. Fig. 4a represents Nyquist plots of different IL-2 concentrations ranged from 1-1,000 pg/ml spiked in human serum in which only slight shift of the impedance spectra curves within a narrow range of around 3 kΩ were observed from different IL-2 concentrations. Fig. 4b represents a relationship between normalized R_{ct} values and different IL-2 concentrations in which no significant change in relative R_{ct} values at all IL-2 concentrations was observed. These results indicate that this system is preferentially interact to TNF-α over IL-2 and therefore suggesting the system specificity toward TNF-α.

4. Conclusions

A method to produce highly sensitive and specific biosensor for the detection of TNF-α from non-diluted serum has been described. The depletion of serum background reduces the effect of background interference therefore enables the sensor matrix to specifically capture the target molecule, in this case, TNF-α. The highly sensitive signal of TNF-α was successfully detected using EIS technique in which the CSGM electrodes were employed for impedance spectra recordings. This method is able to achieve 1 pg/ml LOD and able to detect TNF-α at a dynamic range of 1-1000 pg/ml from non-diluted serum samples. The successful outcomes of this study indicate the proof of system feasibility which can be employed for further development of

microfluidic implementation and automation system for TNF-α biosensor POC application. In addition, the concept built by the present work also provides a guideline for biosensors development that is able to detect biomolecules directly from non-diluted serum.

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References

Arya, S.K., Pui, T.S., Wong, C.C., Kumar, S., Rahman, A.R.A., 2013. Effects of the electrode size and modification protocol on a label-free electrochemical biosensor. Langmuir 29, 6770–6777.

Baker, B.R., Lai, R.Y., Wood, M.S., Doctor, E.H., Heeger, A.J., Plaxco, K.W., 2006. An electronic , aptamer-based small-molecule sensor for the rapid, label-free detection of cocaine in adulterated samples and biological fluids. J. Am. Chem. Soceity 128, 3138–3139.

Bao, B., Chen, Y., Zhang, L., Lin, Y., Xu, N., Wang, X., Liu, J., Qu, W., 2013. Momordica charantia (bitter melon) reduces obesity- associated macrophage and mast cell infiltration as well as inflammatory cytokine expression in adipose tissues. PLoS One 8, e84075.

Battaglia, T.M., Masson, J., Sierks, M.R., Beaudoin, S.P., Rogers, J., Foster, K.N., Holloway, G.A., Booksh, K.S., 2005. Quantification of cytokines involved in wound healing using surface plasmon resonance. Anal. Chem. 77, 7016–7023.

Bradley, J.R., 2008. TNF-mediated inflammatory disease. J. Pathol. 214, 149–160.

Cesaro-tadic, S., Dernick, G., Juncker, D., Buurman, G., Kropshofer, H., Michel, B., Delamarche, E., 2004. High-sensitivity miniaturized immunoassays for tumor necrosis factor α using microfluidic systems. Lab Chip 4, 563–569.

Coates, L.C., Marzo-ortega, H., Bennett, A.N., Emery, P., 2010. Anti-TNF therapy in ankylosing spondylitis: insights for the clinician. Ther. Adv. Musculoskelet. Dis. 2, 37–43.

Cottier, K., Wiki, M., Voirin, G., Gao, H., Kunz, R.E., 2007. Label-free highly sensitive detection of (small) molecules by wavelength interrogation of integrated optical chips. Sensors Actuators B Chem. 91, 241–251.

Dong, J., Zhao, H., Xu, M., Ma, Q., Ai, S., 2013. A label-free electrochemical impedance immunosensor based on AuNPs/ PAMAM-MWCNT-Chi nanocomposite modified glassy carbon electrode for detection of Salmonella typhimurium in milk. Food Chem. 141, 1980–1986.

Du, Y., Chen, C., Yin, J., Li, B., Zhou, M., Dong, S., Wang, E., 2010. Solid-state probe based electrochemical aptasensor for cocaine: a potentially convenient , sensitive , repeatable , and integrated sensing platform for drugs. Anal. Chem. 82, 1556–1563.

Feldmann, M., Brennan, F.M., Maini, R.N., 1996. Role of cytokines in rheumatoid arthritis. Annu. Rev. Immunol. 14, 397–440.

Grieshaber, D., MacKenzie, R., Vörös, J., Reimhult, E., 2008. Electrochemical biosensors - sensor principles and architectures. Sensors 8, 1400–1458.

Homola, J., Yee, S.S., Gauglitz, G., 1999. Surface plasmon resonance sensors: review. Sensors Actuators B Chem. 54, 3–15.

Hu, Y., Zuo, P., Ye, B., 2013. Label-free electrochemical impedance spectroscopy biosensor for direct detection of cancer cells based on the interaction between carbohydrate and lectin. Biosens. Bioelectron. 43, 79–83.

Idriss, H.T., Naismith, J.H., 2000. TNFα and the TNF receptor superfamily: structure-function relationship(s). Microsc. Res. Tech. 50, 184–195.

Kar, P., Pandey, A., Greer, john J., Shankar, K., 2012. Ultrahigh sensitivity assays for human cardiac troponin I using TiO 2 nanotube arrays. Lab Chip 12, 821–828.

Kenison, D.C., Elsasser, T.H., Fayer, R., 1990. Radioimmunoassay for bovine tumor necrosis factor: concentrations and circulating molecular forms in bovine plasma . J. Immunoassay 11, 177–198.

Kongsuphol, P., Arya, S.K., Wong, C.C., Polla, L.J., Park, M.K., 2014. Coiled-coil peptide based sensor for ultra-sensitive thrombin detection. Biosens. Bioelectron. 55, 26–31.

Krebs, H.A., 1950. Chemical composition of blood plasma and serum. Annu. Rev. Bochemistry 19, 409–430.

Kumar, S., Kumar, S., Anand, P., Agrawal, V.V., John, R., Maji, S., Malhotra, B.D., 2013. Microfluidic-integrated biosensors: Prospects for point-of-care diagnostics. Biotechnol. J. 8, 1267–1279.

Kyle, S., Chandler, D., Griffiths, C.E.M., Helliwell, P., Lewis, J., Mcinnes, I., Oliver, S., Symmons, D., Mchugh, N., 2005. Guideline for anti-TNF-α therapy in psoriatic arthritis. Rheumatology 44, 390–397.

Lao, Y.-H., Peck, K., Chen, L.-C., 2009. Enhancement of aptamer microarray sensitivity through spacer optimization and avidity effect. Anal. Chem. 81, 1747–1754.

Lasia, A., 1999. Electrochemical impedance spectroscopy and its applications, in: Conway, B.E., Bockris, J., White, R.E. (Eds.), Modern Aspects of Electrochemistry. Kluwer Academic/Plenum Publishers, New York, pp. 143–248.

Law, W.-C., Yong, K.-T., Baev, A., Prasad, P.N., 2011. Sensitivity improved surface plasmon resonance biosensor for cancer biomarker detection based on plasmonic enhancement. ACS Nano 5, 4858–4864.

Lee, T.M., 2008. Over-the-counter biosensors: past, present, and future. Sensors 8, 5535–5559.

Leister, K.P., Huang, R., Goodwin, B.L., Chen, A., Austin, C.P., Xia, M., 2011. Two high throughput screen assays for measurement of TNF-α in THP-1 cells. Curr. Chem. Genomics 5, 21–29.

Li, J., Huang, J., Wu, B., Chen, Q., 2005. Effects of propyl gallate on interaction between TNF-α and sTNFR-I using an affinity biosensor. Acta Pharmocologica Sin. 26, 1212–1216.

Li, T., Fan, Q., Liu, T., Zhu, X., Zhao, J., Li, G., 2010. Detection of breast cancer cells specially and accurately by an electrochemical method. Biosens. Bioelectron. 25, 2686–2689.

Liu, Y., Kwa, T., Revzin, A., 2012. Biomaterials simultaneous detection of cell-secreted TNF-α and IFN-γ using micropatterned aptamer-modified electrodes. Biomaterials 33, 7347–7355.

Liu, Y., Zhou, Q., Revzin, A., 2013. An aptasensor for electrochemical detection of tumor necrosis factor in human blood. Analyst 138, 4321–4326.

Luo, X., Xu, M., Freeman, C., James, T., Davis, J.J., 2013. Ultrasensitive label free electrical detection of insulin in neat blood serum. Anal. Chem. 85, 4129–4134.

Medzhitov, R., 2008. Origin and physiological roles of inflammation. Nature 454, 428–435.

Piliarik, M., Bocková, M., Homola, J., 2010. Surface plasmon resonance biosensor for parallelized detection of protein biomarkers in diluted blood plasma. Biosens. Bioelectron. 26, 1656–1661.

Pui, T.S., Kongsuphol, P., Arya, S.K., Bansal, T., 2013. Chemical Detection of tumor necrosis factor (TNF-α) in cell culture medium with label free electrochemical impedance spectroscopy. Sensors Actuators B Chem. 181, 494–500.

Qi, H., Shangguan, L., Li, C., Li, X., Gao, Q., Zhang, C., 2013. Sensitive and antifouling impedimetric aptasensor for the determination of thrombin in undiluted serum sample. Biosens. Bioelectron. 39, 324–328.

Qureshi, A., Niazi, J.H., Kallempudi, S., Gurbuz, Y., 2010. Label-free capacitive biosensor for sensitive detection of multiple biomarkers using gold interdigitated capacitor arrays. Biosens. Bioelectron.

Rubin, R.L., Wall, D., Konstantinov, K.N., 2014. Electrochemical biosensor for quantitation of anti-DNA autoantibodies in human serum. Biosens. Bioelectron. 51, 177–183.

Van Deventer, S.J., 1997. Tumour necrosis factor and Crohn's disease. Gut 40, 443–448.

Vasan, A.S.S., Mahadeo, D.M., Doraiswami, R., Huang, Y., Pecht, M., 2013. Point-of-care biosensor system. Front. Biosci. (Shcolar Ed. 5, 39–71.

Weng, S., Chen, M., Zhao, C., Liu, A., Lin, L., Liu, Q., Lin, J., Lin, X., 2013. Label-free electrochemical immunosensor based on $K_3[Fe(CN)_6]$ as signal for facile and sensitive determination of tumor necrosis factor-alpha. Sensors Actuators B. Chem. 184, 1–7.

Zhang, J., Pan, X., Li, N., Li, X., Wang, Y., Liu, X., Yin, X., Yu, Z., 2014. Grape seed extract attenuates arsenic-induced nephrotoxicity in rats. Exp. Ther. Med. 7, 260–266.

Table 1 comparison of the EIS-based TNF-α biosensors

Sensing matrix	Media	LOD	Detection range	Ref.
Antibody immobilized on magnetic beads to deplete serum background and capture TNF-α	un-diluted serum	1 pg/ml	1-1000 pg/ml	present work
Aptamer	blood	10 ng/ml	10-100 ng/ml	(Liu et al., 2013)
Aptamer	culture media without serum	10 ng/ml	10-100 ng/ml	(Liu et al., 2012)
Antibody ($K_3[Fe(CN)_6]$ was utilized for signal detection)	PBS	20 pg/ml	0.02-34 ng/ml	(Weng et al., 2013)
Antibody immobilized on gold nano-particles (alkaline phosphate was utilized for signal detection)		10 pg/ml	0.02-200 ng/ml	Yin et al., 2011 BB
Antibody (poly(guanine) immobilized on silica nano particles were utilized for signal detection)	PBS	50 pg/ml	0.1-100 ng/ml	Wang et al., 2006 Anal.Chem
Antibody	culture media (with 10% serum)	1 pg/ml	1-100 pg/ml	(Pui et al., 2013)
Antibody	PBS	25 pg/ml	0.025-25ng/ml	(Qureshi et al., 2010)

Figure legends

Fig 1. Scheme of the TNF-α system sensor. (a) Serum background was removed using magnetic beads coupled albumin and IgG antibodies. (b) The background removed sample was transferred to a new tube and TNF-α was then captured using magnetic bead coupled TNF-α antibody. (c) The captured TNF-α was eluted from the magnetic beads using 2% SDS at 63°C and (d) the magnetic beads was separated out of the eluted sample. (e) Impedance spectra of the eluted sample were then analyzed using EIS technique in which CSGM electrode was employed as a platform for TNF-α adsorption and for EIS analysis. R, W, and C labeled on CSGM electrodes denote reference, working, and counter electrodes respectively. Nyquist plots represent impedance spectra recorded from (i) blank chip and (ii) eluted sample of 1 pg/ml TNF-α spiked in human serum.

Fig 2. Nyquist plots (a) and R_{ct} values (b) of elute samples of 1-1000 pg/ml TNF-α spiked in PBS. (a) Nyquist curves show increase in R_{ct} values (semicircle diameter) upon increasing in TNF-α concentrations. (b) Relationship between normalized R_{ct} values and different TNF-α concentrations shows a proportional linear relationship of increasing R_{ct} values upon increasing TNF-α concentrations.

Fig 3. Nyquist plots (a) and R_{ct} values (b) of elute samples of 1-1000 pg/ml TNF-α spiked in human serum. (a) Nyquist curves show increase in R_{ct} values (semicircle diameter) upon increasing in TNF-α concentrations. (b) Relationship between normalized R_{ct} values and different TNF-α concentrations shows a proportional linear relationship of increasing R_{ct} values upon increasing TNF-α concentrations.

Fig. 4. Nyquist plots (a) and R_{ct} values (b) of elute samples of 1-1000 pg/ml IL-2 spiked in human serum. (a) Nyquist curves show only slight shift in R_{ct} values (semicircle diameter) upon

increasing in IL-2 concentrations. (b) Relationship between normalized R_{ct} values and different TNF-α concentrations shows no significant change in R_{ct} values upon increasing IL-2 concentrations.

Highlights

- A technique to detect TNF-α from non-diluted serum is described
- Background interference effect is reduced by removing serum abundance proteins
- TNF-α is captured using magnetic bead coupled antibody and detected using EIS
- CSGM electrode is used to enhance TNF-α detection sensitivity
- The technique provides a 3 step procedures for microfluidic system implementation

Figure 1

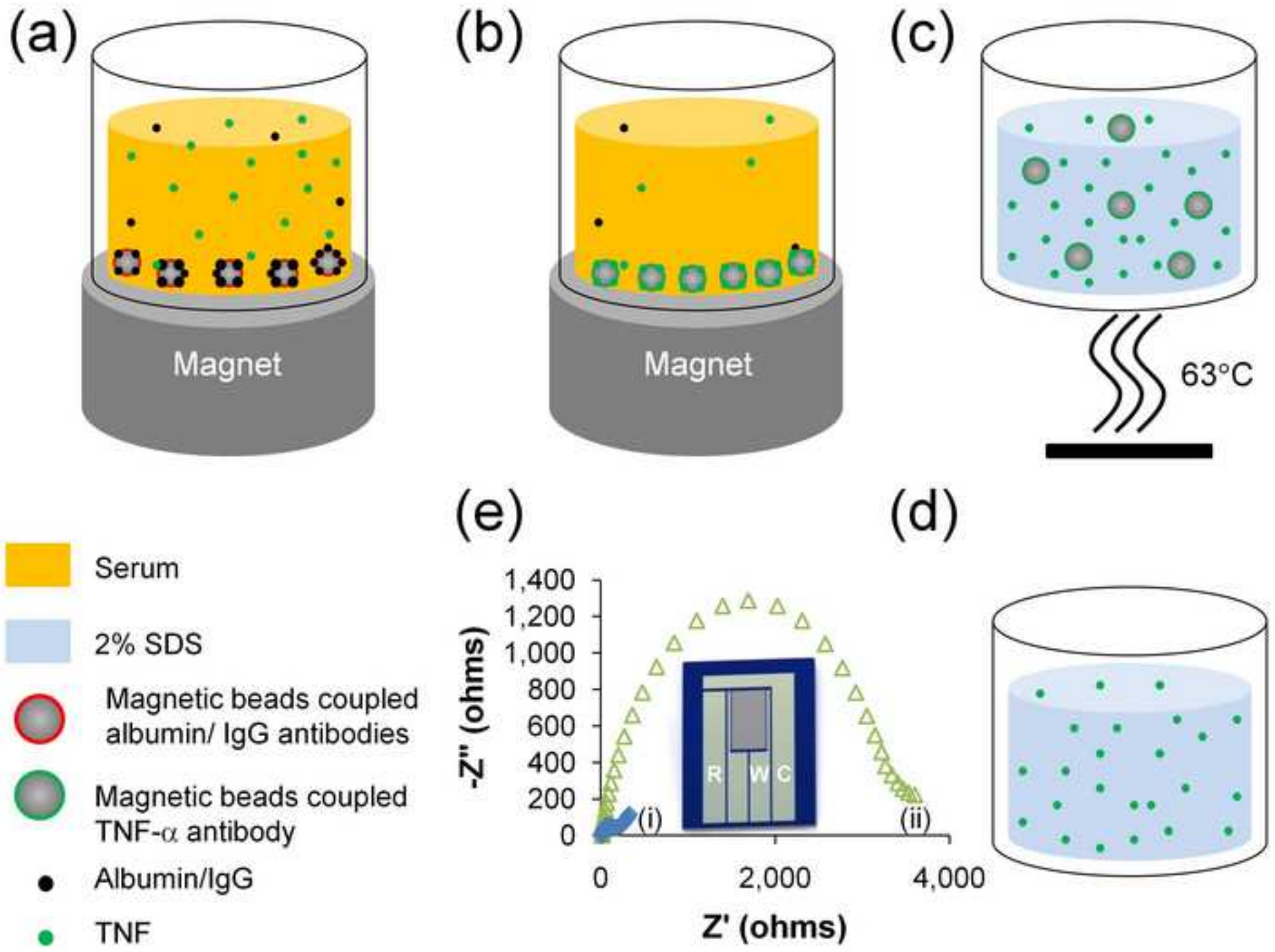

Figure 2

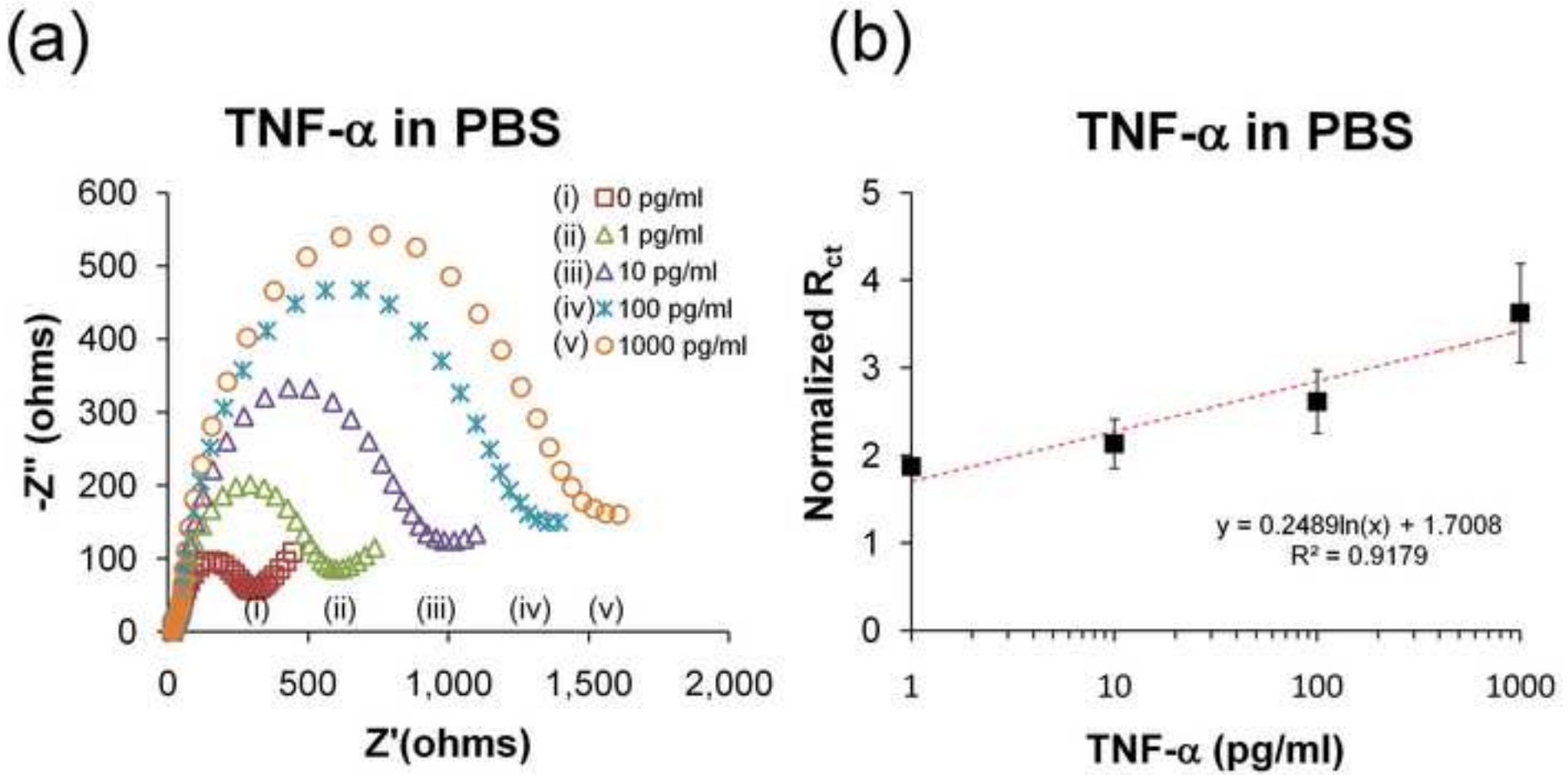

Figure 3

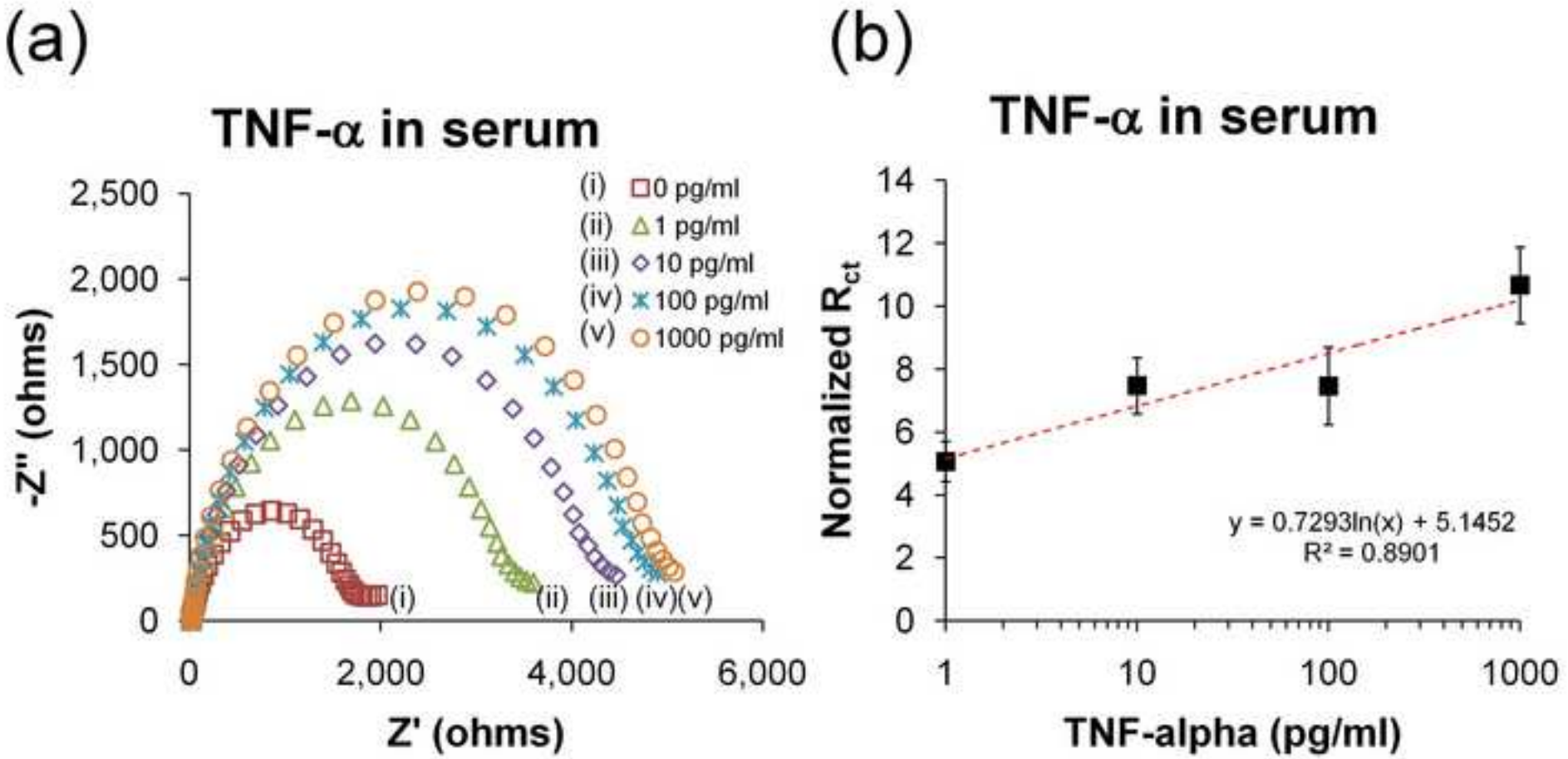

Figure 4