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A New, Sensitive and Disposable Electrochemical Immunosensor Based on Benzaldehyde Side Group Containing Phosphazene Polymer Modified ITO Substrate for Interleukin 1β Detection

İnterlökin 1β Tespiti için Benzaldehit Yan Grup İçeren Fosfazen Polimer ile Modifiye Edilmiş ITO Substrat Temelli Yeni, Hassas ve Tek Kullanımlık Bir Elektrokimyasal İmmünosensör

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ABSTRACT

In this study, a novel electrochemical ultrasensitive immunosensor based on disposable benzaldehyde substituted phosphazene polymer (BSPP) modified ITO electrode was developed for interleukin 1β (IL 1β) detection. Aldehyde side groups containing phosphazene polymer (BSPP) synthesized via ring opening polymerization method. These aldehyde groups provided anchoring points for anti-IL 1β antibodies. The production process of the proposed immunosensor was monitored by electrochemical techniques like Electrochemical Impedance Spectroscopy (EIS) and Cyclic Voltammetry (CV). In addition, these fabrication steps were characterized by utilizing Scanning Electron Microscopy (FE-SEM) and Atomic Force Microscopy (AFM). Moreover, BSPP polymer layer on the polymer coated electrode surface was investigated by using Energy Dispersive X-ray (EDX). The fabricated immunosensor had a low detection limit (9.3 fg/mL) and a wide linear detection range (0.03-7.5 pg/mL). Moreover, it had good reproducibility (1.82%), excellent repeatability (1.56%), good selectivity and high stability. The results of experiments showed that the BSPP polymer was desirable platform for IL 1β antigen detection in clinical diagnosis and practical applications. The applicability of the suggested biosensor was tested by measuring IL 1β level in human serum and the suggested immunosensor had acceptable results for quantitative analysis.

Key Words

Poly(phosphazene), label free immunosensor, electrochemical impedance spectroscopy, interleukin 1β (IL 1β).

ÖZ

Bu çalışmada, interlökin 1β (IL 1β) tespiti için tek kullanımlık benzaldehit ikameli fosfazen polimeri (BSPP) ile modifiye edilmiş ITO elektrot temelli yeni bir elektrokimyasal ultrahassas immünosensör geliştirilmiştir. Aldehit yan grupları içeren fosfazen polimeri (BSPP) halka açma polimerizasyonu yöntemi ile sentezlenmiştir. Bu aldehit grupları, anti-IL 1β antikorları için bir bağlantı noktaları sağlamıştır. Önerilen immünosensörün üretim süreci, Elektrokimyasal Empedans Spektroskopisi (EIS) ve Döngüsel Voltammetri (CV) gibi elektrokimyasal tekniklerle izlendi. Ek olarak, bu üretim aşamaları, Taramalı Elektron Mikroskobu (FE-SEM) ve Atomik Kuvvet Mikroskobu (AFM) kullanılarak karakterize edildi. Ayrıca, polimer kaplı elektrot yüzeyindeki BSPP polimer tabakası, Enerji Dağıtıcı X-ışını (EDX) kullanılarak araştırıldı. Üretilen immünosensör düşük bir tespit sınırına (9.3 fg / mL) ve geniş bir doğrusal tayin aralığına (0.03-7.5 pg / mL) sahiptir. Ayrıca, iyi tekrarlanabilirlik (%1.82), mükemmel tekrarlanabilirlik (%1.56), iyi seçicilik ve yüksek stabiliteye sahipti. Deney sonuçları, BSPP polimerinin, klinik teşhis ve pratik uygulamalarda IL1β antijen tespiti için arzu edilen bir platform olduğunu gösterdi. Önerilen biyosensörün uygulanabilirliği, insan serumunda IL 1β seviyesi ölçülerek test edildi ve önerilen immünosensör, nicel analiz için kabul edilebilir sonuçlara sahipti.

Anahtar Kelimeler

Poli(fosfazen), label free immunosensor, elektrokimyasal impedans spectroskopi, interleukin 1β (IL 1β).

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INTRODUCTION

Rapid and accurate detection of tumor biomarkers is important for early detection of cancer. In recent years, enzyme-linked immunosorbent assay and chemiluminescence immunoassay have been employed for IL 1β detection. These methods require expensive devices and long operation procedure. Because of these disadvantages, a sensitive and rapid method is required for IL 1β biomarker detection. Specific binding between anti-IL 1β antibody and IL 1β antigen is the key strategy for rapid detection of this biomarker by using low cost, sensitive and selective immunosensor. Recently, label-free electrochemical immunosensors are promising tools owing to easy fabrication process and rapid detection of several analytes. The design of a biosensor matrix is important and different types of materials are utilized as immobilization matrices. Polymers have significant roles in the fabrication of electrochemical biosensors. They had a lot of end groups and they provide more antibody loading on the biosensor surface.

Polyphosphazenes are members of the polymer family having an inorganic backbone containing nitrogen and phosphorus atoms. In addition, the advantage of using these polymers is attachment of different substituents to phosphorus backbone. As a result of this attachment, these polymers have unique properties and they can use in different applications such as biomedical studies and drug delivery researches [1]. Furthermore, polyphosphazenes prevent the growth of bacteria and yeast cells and they are biodegradable. The backbone of the polyphosphazenes can be diversified easily by different functional site groups [2]. Therefore, they are promising immobilization matrices for biomolecules in biosensor applications.

Interleukin 1β (IL-1β) is a protein and it has a significant role in several biological functions like immune system. Its cDNA encodes a precursor protein of 269 amino acids. This polypeptide is firstly precursor protein and after that it turns to the 15–20 kDa protein which is responsible for IL 1 activity[3]. IL 1β is usually produced in macrophages, mucosa epithelial cells, acinar and ductal cells of the salivary glands. In addition, IL-1β is a biomarker in lung, colon and breast cancer, oral carcinoma and skin melanomas [4, 5]. For the sensitive determination of IL-1β, different biosensors with different modification strategies and detection techniques have been employed and these biosensors are compared in Table 1A. Krause et al. (2015) fabricated a microfluidic array amplified with magnetic beads and gold nanoparticles (AuNPs) for IL-1β and other cancer biomarkers determination to investigate mucositis risk in cancer patients [6]. A fiber-optic particle plasmon resonance sensor was developed by Chiang et al., and the detection principle was based on molecular binding of IL-1β to anti-IL-1β conjugated AuNPs. The linear range and detection limit of this sensor were 0.05-10 ng/mL and 21 pg/mL, respectively [7]. Baraket et al. (2017) fabricated a biosensor based on eight gold microelectrodes as working electrode placed onto silicon substrates. The linear range and detection limit of this biosensor were 1-15 pg/mL and 0.3 pg/mL, respectively [8].

Electrochemical biosensors are gained interest in electroanalytical applications because of their good sensitivity, high stability, favorable specificity, and repeatability. The working principle of these biosensors is based on specific reaction between the target molecule and biorecognition molecule. As a result of this reaction, an electrical signal forms and this signal is associated to concentration of target molecule. Electrochemical impedance spectroscopy (EIS) is an useful tool to monitor of biosensing system. This method is usually employed for monitoring of modified electrode features during the fabrication procedure. An EIS biosensor system contains a biosensing element, which is immobilized on the working electrode and this biosensing element interacts with the target molecule. In EIS based immunosensors, antibodies are utilized as biorecognition molecules and they detect their antigens. This type immunosensors provide label free detection of target molecules [9, 10]. In addition, EIS based immunosensors are ultrasensitive and low cost devices. The cost of a biosensor is based on utilized strategy during the fabrication [10, 11]. The label-free strategy reduced the cost of immunosensor [12, 13]. For the development of an immunosensor, different ways were utilized for working electrode fabrication.Drop casting, adsorption, electrophoretic deposition, electrochemical deposition are the techniques for biosensor fabrication. The utilized technique and materials increases the stability of the biosensor [14]. Spin-coating is a working electrode fabrication method that based on deposition of fluid on the electrode surface. In this method, uniform, reproducible and thin film is formed on the working electrode[15, 16]. This method is simple, but it is rarely employed for biosensor production.

In this work, a sensitive and selective EIS based immunosensor was developed by using BSPP polymer with benzaldehyde side groups modified disposable ITO sheet for IL 1β biomarker detection. These aldehyde groups were utilized for anchoring of anti-IL 1β antibodies on the ITO sheet. For the modification of ITO sheet, spin-coating method was utilized. After modification of ITO sheets with benzaldehyde substituted poly(phosphazene), aldehyde groups were found on the ITO sheet. These aldehyde groups were attached to amino groups of antibodies directly. Consequently, bioelectrodes were prepared by using simple and easy way. In addition, EIS and cyclic voltammetry methods were employed for monitoring of electrode fabrication and immunosensor analytical performance. The repeatability, reproducibility and real serum applications were performed to investigate the performance of the suggested immunosensor. Furthermore, the accuracy of the proposed immunosensor was examined via standard addition method.

MATERIALS and METHODS

Materials and Apparatus

Phosphonitrilic chloride trimer ($P_3N_3Cl_6$, 97%), 4-hydroxybenzaldehyde (98%), Triethylamine (TEA, ≥99%), Aluminum Chloride (AlCl3, 99%) and Sodium metal rod, tetrahydrofuran (anhydrous, ≥99.9%), Acetone (≥99.9%), Chloroform, (max. 0.003% H_2O), Methanol (≥99.9%) and Hexane (95%) were obtained from Sigma-Aldrich and were employed in synthesis and purification processes. $K_4[Fe(CN)_6]$ and $K_3[Fe(CN)_6]$, KCl, KH_2PO_4, K_2HPO_4 were from Sigma-Aldrich. Clear glass prescored ampoules (10 mL x 108 mm) were purchased from Wheaton® company. Indium tin oxide (ITO) coated PET sheets (2 mm x 20 mm, 60 Ω/cm2), Anti-IL 1β antibody, and IL 1β antigen were supplied by Sigma-Aldrich. These protein solutions were prepared using phosphate buffer (PBS 50 mM, pH 7.4).

Chemical and Morphological Analyses

Electrochemical analyses (CV and EIS) were performed via Gamry Potentiostat/Galvanostat Reference 1000 device. ITO sheet working electrode (2*20 mm), a platinum wire counter electrode, and an Ag/AgCl reference electrode were used for electrochemical experiments. CVs were taken in a potential range from 0 to 500 mV at a scan rate 100 mV/s. EIS analyses were performed from 50 kHz to 0.05 Hz. Proton nuclear magnetic resonance (1H NMR) spectra were taken at a Varian UNITY INOVA 500. Fourier Transform Infrared (FTIR) analyses were taken by utilizing a Bruker Vertex 70 device in the range of 4000–400 cm^{-1}. Dispersive Raman analyses were performed by using a Thermo Scientific DXR-Raman spectrometer with a 780-nm excitation laser. The morphological characterizations of the suggested immunosensor were made by using AFM, SEM and energy dispersive X-ray (EDX) devices. SEM images were recorded utilizing Scanning electron microscope (QUANTA FEG-250) with low vacuum detector. AFM images were recorded utilizing an Ambient AFM, NanoMagnetic Instrument. The images were taken in tapping AFM mode.

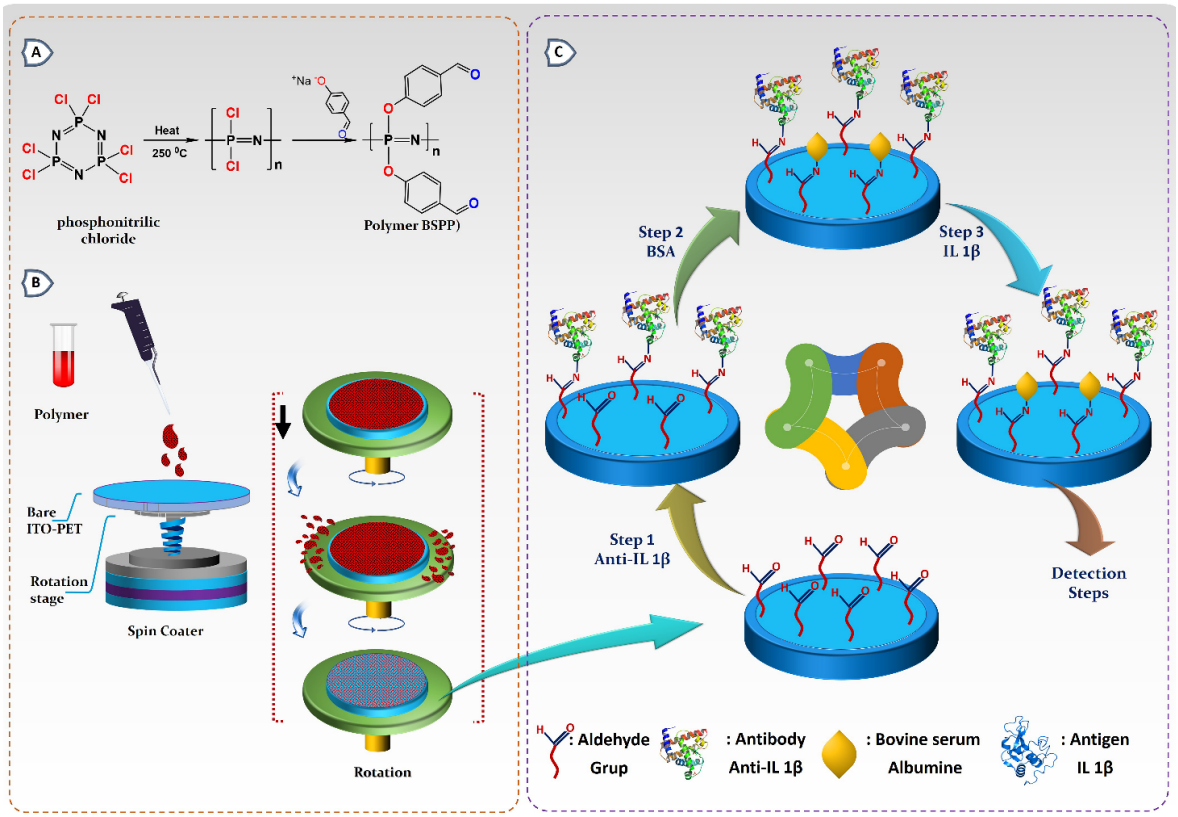
Polymer Synthesis and Preparation of Modified ITO electrode immunosensor

Benzaldehyde side groups containing polymer (BSPP) were synthesized (Scheme 1) and characterized according to our previous report [14]. The chemical characterizations results are given below.

FT-IR (ATR, cm^{-1}): 2832 ($-CH_2$); 1695 (C=O); 1598, 1501(aromatic); 1195 (P=N); 1155 (P-O-C); 920 (P-O-Ph); 830. Raman (λlaser= 780 nm, cm^{-1}): 3073, 3000; 1696 (C=O); 1598, 1509 (aromatic); 1233, 1168 (P=N); 1010 (P-O-Ph); 718(PN). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7,19ppm (Ha; 4H, ortho); 7,48ppm (Hb; 4H, meta); and 9,66ppm (Hc; 1H, CHO). ^{31}P NMR (DMSO-D6, 202.4 MHz): -5.3 ppm.

Scheme 1 contains the polymer synthesis steps, preparation of BSPP polymer coated ITO electrodes and the fabrication protocol of IL 1β immunosensor.

Firstly, the disposable ITO electrodes were sonicated in ethanol, soap solution and ultrapure water, respectively. After that, polymer BSPP was solved in dry THF solvents utilizing ultrasonic bath for 15 minutes. Then, polymer BSPP was dropped on to the clean ITO sheet surface and spinned at 1000 rpm for 60 seconds. After this spin coating process, the polymer BSPP modified ITO electrode was washed with ultrapure water. Next, an anti-IL 1β antibody solution was utilized for immersing the polymer BSPP modified ITO electrode and incubated there for 1 h. In this step, antibodies immobilized electrode surface because of the existence of aldehyde groups of the polymer BSPP. After rinsing of anti-IL 1β antibody attached ITO-PET electrode with ultrapure water. BSA solution was employed to block remaining aldehyde groups. Then, the prepared immunoelectrodes were immersed in various concentrations of IL 1β solutions and incubated there for 1 hour.



Sheme 1. (A) Synthesis pathway of Polymer BSPP, (B) Preparation of BSPP polymer coated ITO electrodes and (C) fabrication protocol of IL 1β immunosensor.

Real Serum Sample Measurements

Human serum samples were supplied by the Tekirdağ Namık Kemal University Faculty of Medicine. These serum samples were examined by using the suggested biosensor after a 20 fold dilution with PBS. The accuracy of the suggested biosensor was studied by addition of IL 1β antigen to the diluted human serum samples.

RESULTS and DISCUSSION

Chemical Characterization of the ITO Electrode Surface

Benzaldehyde substituted polymer (BSPP) were synthesized by reaction sequences with ring opening polymerization and replacement reactions. These reaction stages are shown in Scheme 1. The chemical structure of (BSPP) was monitored with different spectral methods (FTIR, RAMAN, 1H and 31P NMR) to display the BSPP synthesis procedure and spectroscopic results of this synthesis are illustrated our previous report [17].

The chemical bonds between BSPP polymer coated ITO substrate and anti- IL 1β antibody was proved by using FTIR and DXR-RAMAN spectroscopic techniques.

The chemical bonds between BSPP polymer coated ITO substrate and anti- IL 1β antibody was proved by using FTIR and DXR-RAMAN spectroscopic techniques. The FTIR spectra of polymer BSPP layer was taken after spin-coating procedure (green line) and after anti-IL 1β antibody binding on the ITO-PET surface (purple line) are displayed in Fig. 1A and 1B, respectively. As shown in Fig. 1A, the aldehyde substituted BSPP functionalized ITO electrode surface had the two absorption peaks at 1190 cm^{-1} and 830 cm^{-1} that proved the presence of P=N and P-N bonds in polymer [18-21]. The strong signal observed around 1695 cm^{-1} was proved that C=O stretching vibration of aldehyde side groups of polymer BSPP [10]. In addition, the peak around at 920 cm^{-1} was indicated the P-O-Ph stretching vibration of side groups [22, 23]. The basic functional groups of protein structure are -NH and -OH bands (3000–3700 cm-1). As seen red line in Fig. 1B, there were broad and intense bands for amide I at ~1648 cm^{-1} and for amide II at ~1542 cm^{-1}, and this showed the anchoring of the anti- IL 1β antibody to polymer BSPP [17, 24]. The chemical structure of polymer BSPP coated ITO-PET electrode and anti-IL 1β antibody attached ITO substrate was investigated via

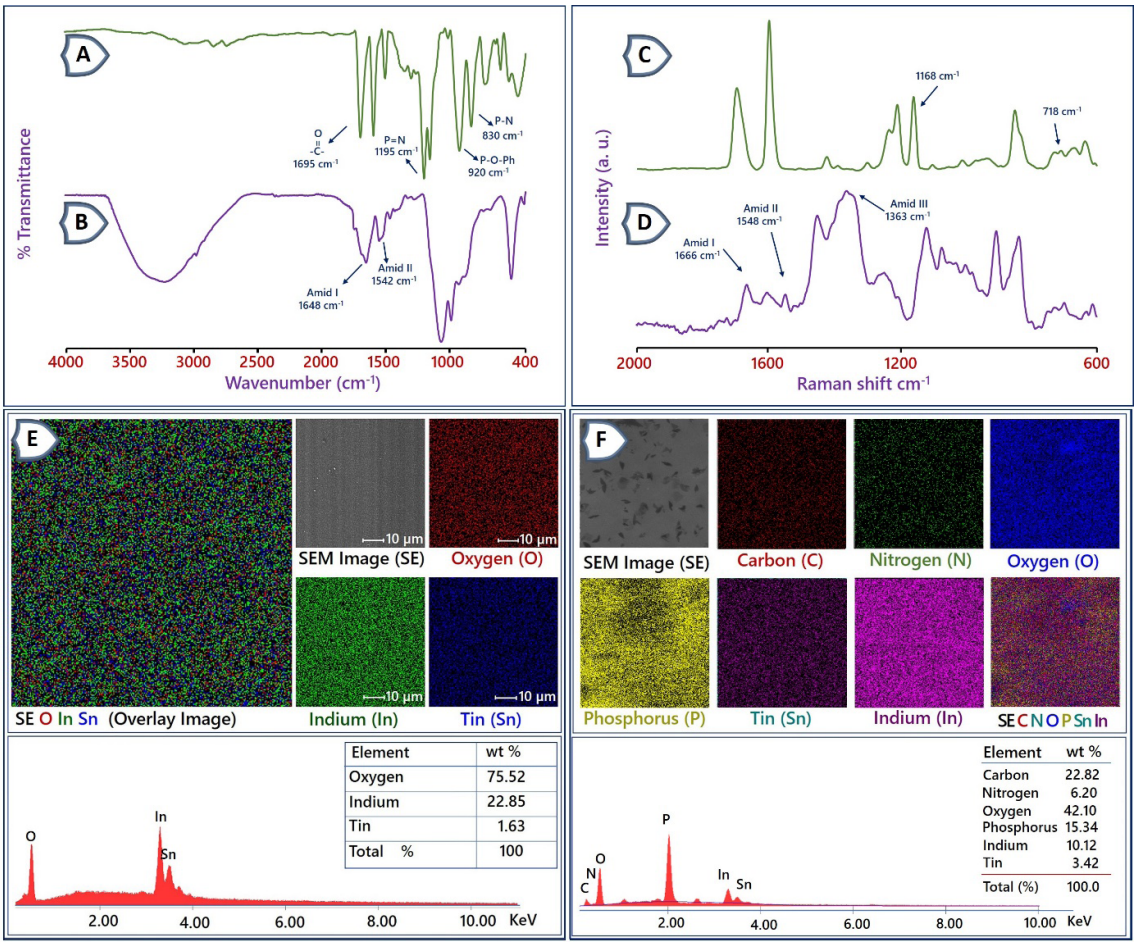


Figure 1. FTIR and Raman spectra of polymer BSPP (A and C) and anti- IL 1β antibody immobilized subatrate (B and D) and SEM-EDX elementel spectra and mapping of bare and BSPP coated electrodes.

Raman spectral method (Fig. 1C and 1D). The chemical structure of BSPP and the protein structure are mostly monitored by utilizing Raman spectroscopy [25-27]. Apart from FTIR spectroscopy, the binding process was monitored via Raman Spectroscopy. The Raman spectra showed that the -PNP- and -PN- bonding stretching vibrations of polymer BSPP backbone were observed at 718 cm^{-1} and 1168 cm^{-1} [18]. Raman spectroscopy is utilized to show amide bonds in protein structure. The bands of amide I, II and III bonds are found between 1650–1680 cm^{-1}, 1480-1570 cm^{-1} and 1235-1300 cm^{-1}, respectively [28]. The amide I bond was seen at 1666 cm^{-1} [29]. The amide II band is usually weak in Raman spectra, but the amide III band is generally more apparent [30]. As illustrated in Fig. 1D, amide II and amide III bonds were monitored at 1548 and 1363 cm^{-1}, respectively [31, 32].

In addition, clean ITO sheet and P-PHP polymer functionalized ITO sheet were investigated by Energy Dispersive X-Ray (SEM-EDX) for elemental mapping measurement. Fig. 1E and 1F inset show the SEM image of the ITO sheet was utilized in mapping analysis. Fig. 1E and 1F show the EDX spectrum of the clean electrodes and polymer BSPP coated electrode. The Oxygen (O), Indium (In) and Tin (Sn) peaks from ITO layer on PET (polyethyleneterefталat) are seen at 0.5, 3.28 and 3.44 keV, respectively (Fig. 1E) [4, 33, 34]. As seen in Fig. 1F, EDX-mapping spectra of polymer BSPP coated electrode included Carbon (C), Oxygen (O), Nitrogen (N), Phosphorous (P), Indium (In) and Tin (Sn) elements. The phosphorous and nitrogen signal at 2.01 and 0.38 keV proved to presence polymer BSPP. This image displayed that the P and N atoms were homogeneous distributed on the electrode surface.

Electrochemical Characterization of the Bioelectrode

EIS is an electroanalytical method to monitor electrochemical changes formed on the electrode surface [35, 36]. Fig. 2A shows the EIS spectra of different stages in immunosensor fabrication process.

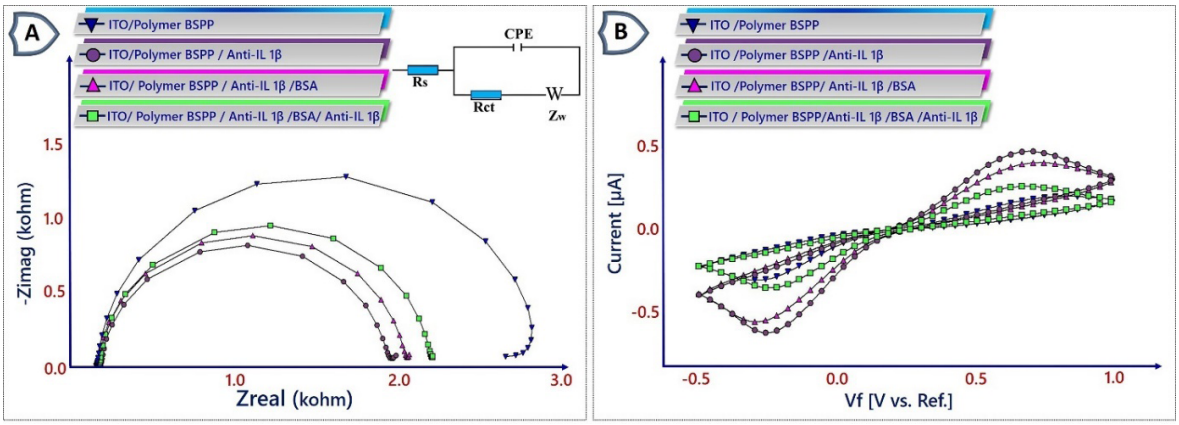


Figure 2. EIS spectra and cyclic voltammograms of the suggested biosensor.

The EIS data are fitted to a Randles equivalent circuit (inset in Fig. 2A) that includes the electrolyte solution resistance (Rs), the charge transfer resistance (Rct), the constant phase element (CPE) and Warburg impedance (W).In the EIS spectra, the semicircle diameter corresponds to the Rct parameter of redox conversion of the electroactive couple $[Fe(CN)_6]3^{-/4-}$ on the ITO-PET surface at certain potential [37].

In this study, EIS and CV were utilized for characterization of immunosensor fabrication protocol. Figure 2A shows EIS spectra of the modification process. After polymer BSPP film coating on the disposable ITO sheet, a large semicircle diameter and also high Rct value was obtained. The high Rct value was the evidence of non-conductive layer formation.

After antibody immobilization process, decreases were seen in semicircle diameter of Nyquist plot and Rct value. Next, in the BSA blockage step an increase was monitored in Rct value due to BSA immobilization on the remaining aldehyde groups. As expected, Rct was further increased due to effective interaction between anti-IL 1β antibody and IL 1β antigen. The changes in the Rct values confirmed the fabrication protocol of the immunosensor. Figure 2B illustrates the CVs of the proposed immunosensor. Obviously, low peak current was

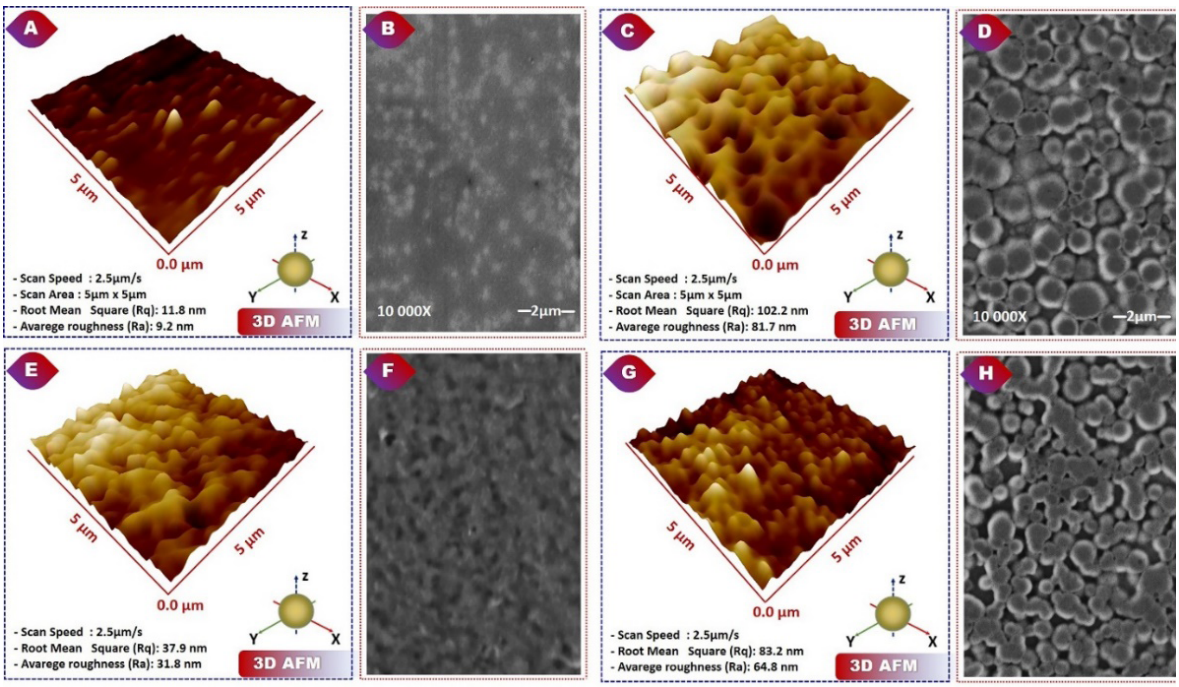


Figure 3. SEM and AFM images of ITO electrodes of the suggested biosensor.

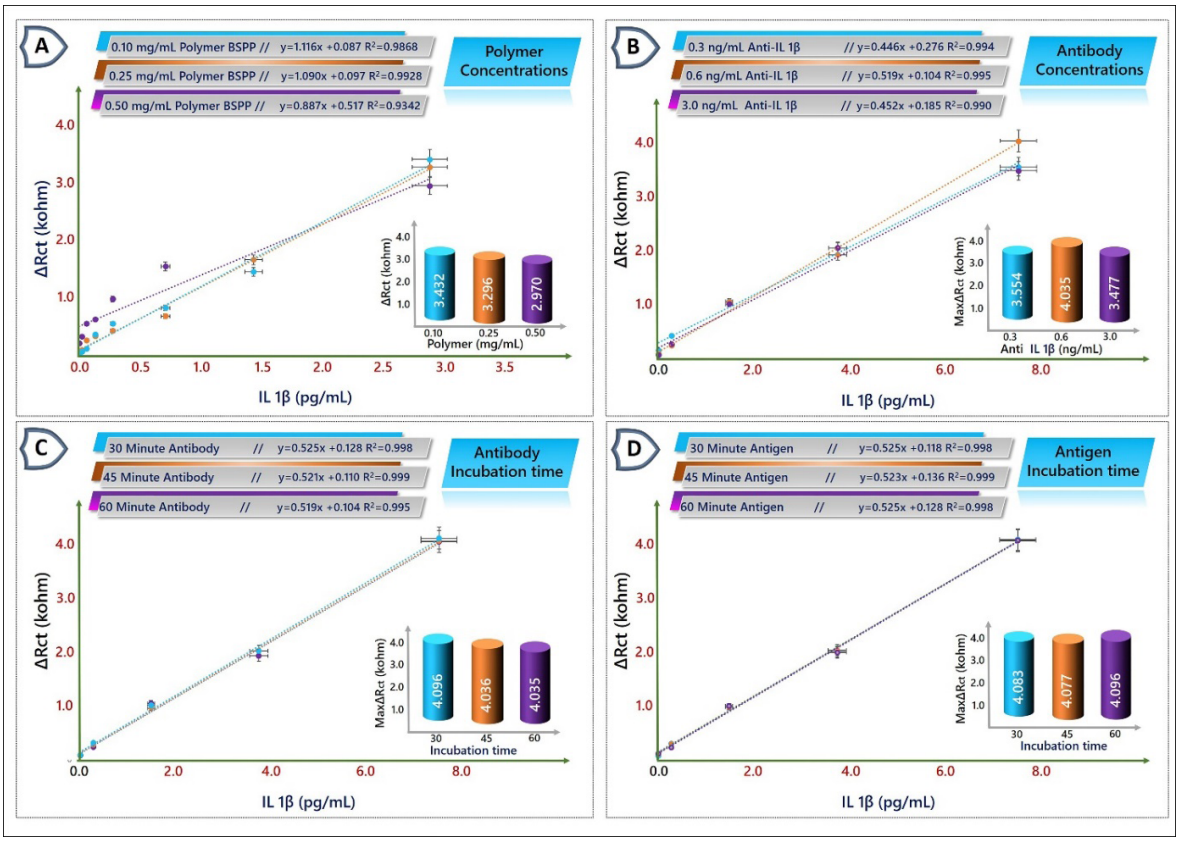


Figure 4. Optimization results (A) BSPP quantity, (B) anti- IL 1β concentration, (C) anti- IL 1β incubation duration, (D) IL 1β antigen incubation duration.

seen after electrode coating with BSPP polymer modification due to nonconductivity property of polymer. The peak currents were increased after the anchoring of anti-IL 1β antibodies. At the BSA blockage stage, decreases were seen in peak currents owing to blocking free aldehyde groups. Protein molecules inhibited electron transfer between electrode and electrolyte solution. Furthermore, the peak currents were decreased after attaching IL 1β antigens thanks to the immunocomplexes formation. These findings proved the effective construction of the proposed biosensor.

Morphological characterization of the suggested Biosensor

Morphological characterization of the proposed immunoelectrodes was carreid out via SEM and AFM monitoring. After the polymer BSPP modification (Fig. 3A and 3B), the electrode surface appeared like spherical grains and a uniform film was coated on the ITO electrode. Ra value of BSPP coated electrode was found as 9.2 nm. In addition, as seen in Figure 3A, the ITO surface had densely porosity that proved a uniform ITO surface was formed for immobilizing of IL 1β antibodies. Figure 3C and 3D show the successful immobilization of anti- IL 1β antibodies on the ITO substrate. As observed in Fig. 3D, antibodies looked like granules. The Ra value of this step was measured as 81.7 nm (Figure 3C). After BSA immobilization, ITO electrode surface looked like a layer (Figure 3E and 3F). The Ra value of this stage was measured as 31.8 nm. The immuno-interaction between anti-IL 1β antibodies and IL 1β antigens changed the ITO sheet surface (Figure 3G and 3H). In this step, the Ra value was found as 64.8 nm. The variations formed during the fabrication steps showed the successful fabrication.

Optimization of the Immunosensor Conditions

Immobilization matrix concentration, biorecognition element concentration and biomolecules incubation time are significant variables affecting the proposed immunosensor performance. Three different polymer concentrations were tried. As seen in Figure 4A, higher concentration of polymer BSPP caused low signals and maximum signal was attained when 0.1% polymer was employed. To optimize antibody concentration, three

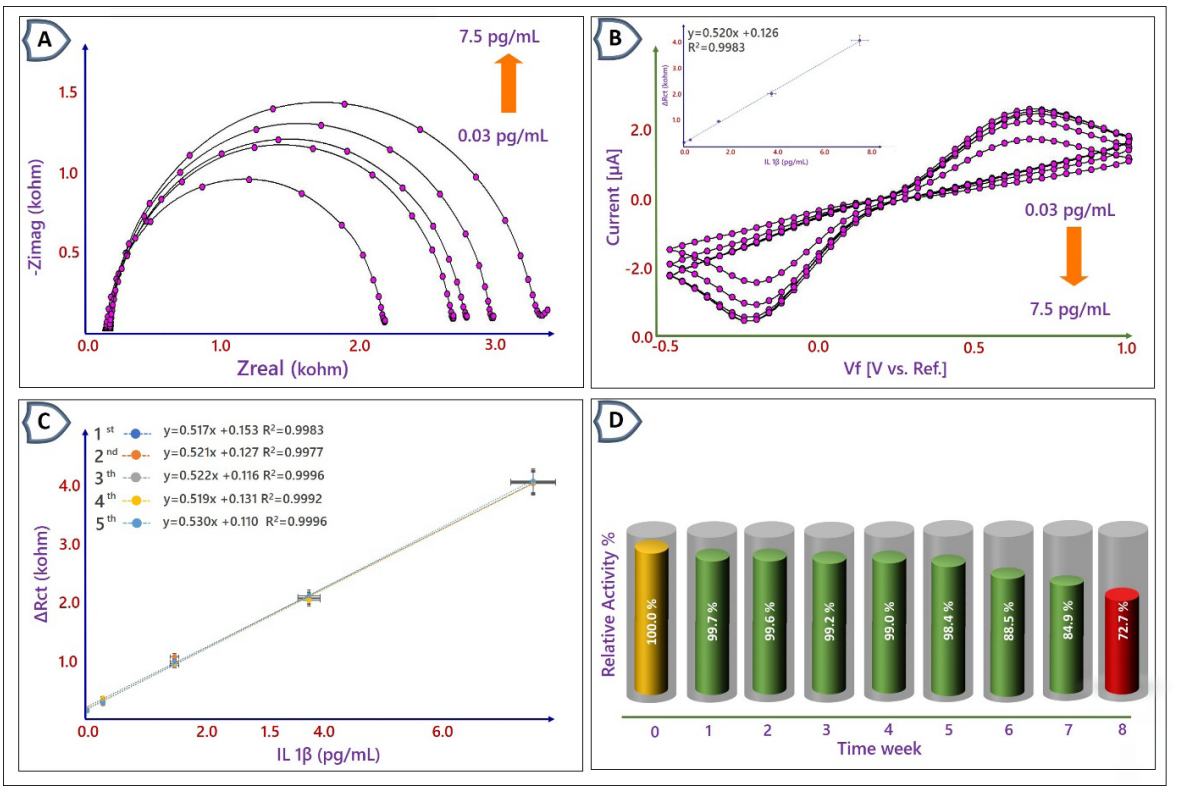


Figure 5. EIS (A) and CV (B) results of the biosensor for the determination of IL 1β biomarker (from 0.03 to 7.5 pg/mL), calibration plot (inset). Reproducibility (C), Storage stability (D) of the biosensor.

different concentration (0.3 ng/mL, 0.6 ng/mL, 3 ng/mL) were utilized. At low concentration of antibody (0.3 ng/mL), a low signal was found. This concentration was not enough for IL 1β detection. When 3 ng/mL anti-IL 1β was employed, a low signal was measured. However, maximum signal was obtained when 0.6 ng/mL anti-IL 1β antibody was used. Because of this, 0.6 ng/mL anti-IL 1β antibody selected optimum level (Figure 4B). In order to optimize antibody and antigen incubation duration, three different incubation durations (30 min, 45 min, 60 min) were studied. The responses were similar at these durations. Maximum signals were obtained at 30 min incubation in antibody and antigen solutions and therefore, 30 min was chosen as optimal incubation time (Figure 4C and 4D). The other experiments were made by using these optimal conditions.

Analytical Performance of the Suggested Biosensor

In this study, EIS technique was used to examine the IL 1β levels on the fabricated biosensor. Figure 5A illustrates the EIS spectra of IL 1β concentrations from 0.03 to 7.5 pg/mL under optimal experimental conditions. As shown in Figure 5A, the diameter of Nyquist plots increased with the enlarged IL 1β concentrations. In contrast to increases in diameters, decreases were seen in peak currents (Figure 5B). The detection and quantification limit were calculated as 9.3 fg/mL and 31 fg/mL, respectively. Figure 5A inset illustrates the calibration plot of the suggested biosensor and the regression equation was y=0.520 [IL 1β] + 0.126 with a correlation coefficient of 0.99.

Repeatability, Reproducibility and Stability

In order to investigate the repeatability of the biosensor, 10 immunoelectrodes were prepared for IL 1β antigen (1.5 pg/mL) detection under same conditions. The relative standard deviation (RSD) of the repeatability test was found as 1.56%. In addition, to test the reproducibility of the biosensor, five biosensors were constructed, and the obtained results were seen in Figure 5C. The RSD value was found as 1.82%, that indicated excellent reproducibility of the biosensor.

For stability test, the proposed immunoelectrodes were stored at 4 oC and their activities were measured every week. As illustrate figure 5D, the impedance response was reduced by 72.7% after 8 weeks storage, demonstrating its long-term stability.

Table 1. A) Biosensors for IL-1β, B) Serum experiments.

A) Biosensor construction step	Linear Range	Limit of Detection	Ref.
Eight gold working microelectrodes /4carboxymethylaryldiazonium / antibodies/BSA	1-15 pg/mL	0.3 pg/mL 0.7 pg/mL	[38]
N_2O plasma modified silicon nanowire /APTES/ glutaraldehyde/DNA	175 fg/mL-175 ng/mL	43.75 fg/mL	[7]
Gold nanoparticles modified fiber optic particle plasmon resonance sensor/antibodies	0.05-10 ng/mL	21 pg/mL	[39]
Dual screen-printed carbon electrodes modified with 4-carboxyphenyl-functionalized double-walled carbon nanotubes/antibodies	0.5-100 pg/mL	0.38 pg/mL	[8]
Polymer BSPP modified ITO electrode/anti-IL-1β/BSA	0.03-7.5 pg/mL	9.3 fg/mL	This work

B) Sample	Found by the biosensor (pg/mL)	Added IL 1ββ amount (pg/mL) Total found	Total Found	% Recovery	% Relative Difference
Human Serum 1	0.41	0.2	0.68	102.60	2.60
Human Serum 2	0.50	0.2	0.76	100.76	0.76
Human Serum 3	0.43	0.2	0.69	100.83	0.83
Human Serum 4	0.34	0.2	0.59	100.31	0.31
Human Serum 5	0.48	0.2	0.73	100.25	0.25

Analysis of Real Human Serum Samples

In order to investigate the practical applicability of the biosensor, the IL 1β antigen concentration was measured in real human serum samples. In addition, standard addition method was utilized to calculate the recovery of the method. As seen Table 1B, the recovery ranges were from 100.25% to 102.60%, that confirmed the applicability of the biosensor for IL 1β determination.

CONCLUSION

To sum up. BSPP polymer was synthesized by using ring opening polymerization and replacement reaction. Then. it was applied to disposable ITO sheet and BSPP modified ITO substrate was used as a working electrode in biosensing system. In this biosensing system. anti-IL 1β antibodi es were employed as biorecognition elements and the basic principle of this biosensing system was based on immuno-interaction between anti-IL 1β antibodies and IL 1β antigens. The immunosensor illustrated a wide linear range of 0.03-7.5 pg/mL. with a low detection limit of 9.3 fg/mL (Signal/Noise=3). As reported in table 1A, the LOD and linear detection range of our developed immunosensor is better than that of other IL-1β sensor reported in the literature. The high sensitivity and wide linear detection range of the suggested immunosensor can be attributed to the uniform polymer film formation. Moreover. it had an excellent reproducibility. good selectivity and long-term stability. The proposed immunosensor had success in human serum for IL 1β detection and it had good recovery (100.25%-102.60%). The obtained results illustrated that this immunosensor was a promising tool for IL 1β detection.

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