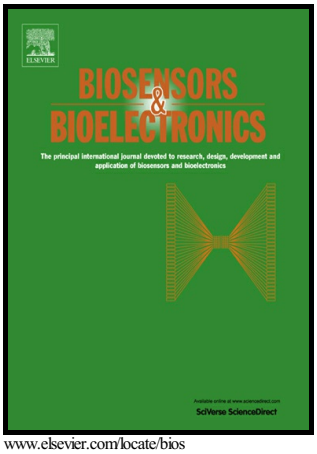

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Title

A highly sensitive and selective impedimetric aptasensor for interleukin-17 receptor A

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Abstract

Interleukin-17 receptor A (IL-17RA) has been recognized as a valuable biomarker for diverse diseases, including autoimmune diseases. In this work, an electrochemical biosensor with great sensitivity and selectivity toward IL-17RA was fabricated using an IL-17RA aptamer (K_d=14.00 nM) for the first time. The aptasensor was manufactured using electrodeposition of gold nanoparticles, and then quantitative detection of IL-17RA was performed based on impedimetry. The developed sensor exhibited a superior analytical performance for IL-17RA with a wide dynamic range of 10 – 10000 pg/mL in buffer and a detection limit of 2.13 pg/mL, which is lower than that of commercially available ELISA kits. In addition, we validated the high specificity of the designed aptasensor to only IL-17RA, which showed good sensitivity even in human serum solution. Furthermore, the detection of the differentiated HL-60 cells expressing IL-17RA was successfully performed. Clinical applicability of the sensor was also demonstrated utilizing neutrophils separated from asthma patients. It is expected that the fabricated aptasensor will become an excellent diagnostic platform for IL-17RA-mediated diseases.

Keywords

IL-17RA; Aptamer; Impedance spectroscopy; Biosensor; Autoimmune disease

1. Introduction

Autoimmune disease is one of the leading causes of death, especially for women in all age groups up to 65 years (Walsh and Rau 2000). When the body's immune system misrecognizes normal substances of the body as foreign materials, autoimmune diseases can occur. There are approximately 80 types of autoimmune disease, and the causative factors and involved substances in each disease vary widely (Rioux and Abbas 2005). Among various factors, interleukin-17 (IL-17) has received substantial attention as a valuable biomarker related to numerous autoimmune diseases such as psoriasis, inflammatory bowel disease, asthma, type 1 diabetes, rheumatoid arthritis, and multiple sclerosis (Duerr et al. 2006; Emamaullee et al. 2009; Graber et al. 2008; Kirkham et al. 2006; Krueger et al. 2007; Molet et al. 2001). In particular, it is known that IL-17A can induce the expression of various inflammatory mediators and regulate the activities of inflammatory cells (Forlow et al. 2001; Linden and Adachi 2002; Sarma et al. 2009). Over-expression of IL-17A in numerous autoimmune diseases is highly associated with the pathogenesis of these diseases (Kolls and Linden 2004; Kotake et al. 1999). IL-17A functions through its receptor, interleukin-17 receptor A (IL-17RA), which plays a critical role among five receptor subunits (Aggarwal and Gurney 2002). Because the cell surface expression level of IL-17RA is strongly correlated with the expression of IL-17A (Gaffen 2009), its over-expression is relevant to several diseases including autoimmune diseases (Alves et al. 2008; Shen et al. 2006; Wang et al. 2013; Zrioual et al. 2008). Therefore, the detection and monitoring of IL-17RA is significant for the diagnosis and prognosis of autoimmune diseases.

To fabricate selective detection platforms for target molecules, antibodies have been traditionally utilized. However, because of some limitations of antibodies such as low stability in harsh conditions, the high cost of development, and restricted screening for only biomolecules, a new antibody substitute is greatly needed (Song et al. 2012). An aptamer is a

target-specific single-stranded DNA, RNA, or peptide molecule generated via the Systematic Evolution of Ligands by Exponential Enrichment (SELEX) technique that has been recognized as a potent alternate to antibodies (Darmostuk et al. 2015; Ellington and Szostak 1990; Tuerk and Gold 1990). It presents diverse advantages compared to antibodies such as small size, chemical synthesis *in vitro*, easy modification, and high stability in various physical and chemical environments, resulting in numerous applications for aptamers in research, prognosis, diagnosis, and therapy (Min et al. 2011; Yuce et al. 2015).

For the accurate detection of target biomarkers, various techniques based on fluorometry, colorimetry, and electrochemistry have been widely employed (Jo et al. 2015a; Jo et al. 2015b). In particular, typical quantitative real-time reverse-transcription polymerase chain reaction or enzyme-linked immunosorbent assay (ELISA) were frequently applied for the detection of IL-17RA (Oliveira et al. 2013; Yano et al. 2012). However, there are some drawbacks to those methods, such as the degradation of RNA, false-positive error, high cost, and blocking errors. On the other hand, electrochemical impedance spectroscopy (EIS) offers rapid, simple, and accurate monitoring of the interaction or reaction of target molecules via sensitive electrochemical signals (Ghoreishi et al. 2015; Jolly et al. 2016). EIS is appropriate for the label-free detection and quantification of target molecules.

[Fig. 1]

In this work, we provide the first design of a highly sensitive and selective biosensor for IL-17RA using the aptamer and the EIS technique. As illustrated in Fig. 1, gold nanoparticles (AuNPs) were electrochemically deposited on the gold electrode, and then the IL-17RA aptamer was immobilized. Each modification process was monitored via cyclic voltammetry (CV), and the calibration curve for IL-17RA was constructed using EIS. Then,

the selectivity of the sensor was demonstrated utilizing several proteins and was also confirmed in human serum-supplemented solution. In addition, the developed sensor was applied for the detection of cells that express IL-17RA, as well as neutrophils from asthma patients.

2. Materials and methods

2.1. Materials

Alumina powders (1, 0.3, and 0.05 μm) and polishing pads were purchased from Bioanalytical Systems (West Lafayette, IN, USA). Gold (III) chloride trihydrate, human serum albumin (HSA), human serum (human male AB plasma), and bovine serum albumin (BSA) were bought from Sigma-Aldrich (St. Louis, MO, USA). The 5'-thiol modified IL-17RA aptamer was obtained from Aptamer Sciences (Seoul, Korea). The extracellular domain of human IL-17RA, interleukin 5 receptor (IL-5R), interleukin 13 receptor (IL-13R), and cluster of differentiation 166 (CD166) were acquired from Sino Biological (Beijing, China). Lysozyme was bought from Bio Basic (Markham, Ontario, Canada).

2.2. Preparation of IL-17RA

To obtain the highly purified IL-17RA, fast performance liquid chromatography (FPLC) was carried out. The powdered form of IL-17RA was dissolved in a phosphate-buffered saline (PBS) buffer (pH 7.4), and then the sample was filtered using a 0.22 μm polyethersulfone membrane filter. The target protein was applied to a HisTrap Ni-NTA affinity column (GE Healthcare, USA) pre-equilibrated with a binding buffer (PBS, pH 7.4, and 10 mM imidazole). The bound IL-17RA was eluted through increasing the concentration of imidazole from 10 to 300 mM with an elution buffer (PBS, pH 7.4, and 300 mM

imidazole). Furthermore, the eluted protein was applied to a Superdex 200 HL gel filtration column (GE Healthcare, USA) pre-equilibrated with storage buffer (PBS, pH 7.4, and 20% (v/v) glycerol). The purity of the protein was evaluated via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and stored at – 80 °C until use.

2.3. Design of the electrochemical sensor for IL-17RA

A gold electrode was chemically washed using a piranha solution, which is a 3:1 mixture of H_2SO_4 and H_2O_2, followed by washing with distilled water. Afterwards, the electrode was consecutively polished with 1, 0.3, and 0.05 μm alumina powder using a polishing pad and washed thoroughly with distilled water. In addition, the electrode was sonicated for 10 min in distilled water to remove traces of alumina powder. All electrochemical detections were performed using a three-electrode system: the gold working electrode, an Ag/AgCl reference electrode in a saturated NaCl solution, and a platinum counter electrode. Electrochemical cleaning was carried out in 100 mM H_2SO_4 by cycling the potential between +0.1 and +1.6 V versus an Ag/AgCl reference at a scan rate of 50 mV/s for 12 cycles. After washing with distilled water and drying with nitrogen gas, the cleaned electrode was immersed in a gold deposition buffer (6 mM $HAuCl_4$ and 100 mM KNO_3) and electrodeposition of the gold nanoparticles was performed by cycling the potential between +0.4 and +1.4 V at a scan rate of 50 mV/s for 20 cycles. To modify the surface of the working electrode, 0.25 μM thiol-modified IL-17RA aptamer and various concentrations of 6-mercapto-1-hexanol were incubated with the electrode under mild shaking for 16 h at room temperature (RT). The electrode was further incubated with 10 mM β-mercaptoethanol for 10 min at RT to eliminate non-specifically bound aptamers and block the non-reacted AuNPs on the surface. Modification of the electrode was monitored via CV and impedimetry using a PARSTAT 2263 (Princeton Applied Research, USA), which were performed in PBS buffer

with 5 mM $[Fe(CN)_6]^{3-/4-}$.

2.4. Electrochemical detection of IL-17RA

For the quantitative analysis, the aptamer-modified gold electrode was incubated with various concentrations (10 pg/mL, 100 pg/mL, 1 ng/mL, and 10 ng/mL) of IL-17RA in PBS solution for 30 min at RT, followed by the measurement of each impedance value. In addition, to demonstrate the stability of this system, the IL-17RA aptamer-immobilized electrode was incubated with diverse concentrations (100 pg/mL, 1 ng/mL, and 10 ng/mL) of IL-17RA supplemented with 10% (v/v) human serum. All detections were performed in PBS solution with 5 mM $[Fe(CN)_6]^{3-/4-}$, and all spectra were recorded within the frequency from 10 kHz to 100 MHz.

2.5. Selectivity test of the aptasensor

The applicability of the designed aptasensor for the detection of IL-17RA was verified via a selectivity test. Various control proteins such as IL-5R, IL-13R, CD166, HSA, BSA, and lysozyme were applied to the electrode at a concentration of 1 ng/mL. Each reaction was performed in PBS buffer for 30 min at RT, and then the Nyquist plots were obtained.

2.6. Differentiation of HL-60 cells to neutrophil-like cells

It is known that neutrophils express IL-17RA (Pelletier et al. 2010), implying that the targeting of IL-17RA allows the detection and the quantification of neutrophils. Therefore, differentiation of HL-60 cells to neutrophil-like cells was carried out according to the general protocol. First, HL-60 cell lines were cultured in Iscove's modified Dulbecco's medium supplemented with 10% (v/v) fetal bovine serum (FBS), 100 U/mL penicillin G, 100 mg/mL

streptomycin, and 50 mM β-mercaptoethanol at 37 °C in a 10% (v/v) CO_2 humid incubator. The differentiation of the HL-60 cells was triggered by the addition of 1.25% (v/v) dimethyl sulfoxide (DMSO) at a concentration of 1 x 10^6 cells/mL. After 3, 5, and 7 days from the treatment of DMSO, the cells were collected on each day. To evaluate the extent of differentiation, the cells were analyzed via fluorescence-activated cell sorting (FACS) using neutrophil markers such as cluster of differentiation 11b (CD11b) and cluster of differentiation 14 (CD14). The cells were stained with a mixture of allophycocyanin (APC)-conjugated CD11b antibodies and peridinin chlorophyll (PerCP)-conjugated CD14 antibodies for 30 min on ice, and fixed with 2% (v/v) paraformaldehyde for 10 min at RT. Then, the differentiation state of the HL-60 cells was measured using a FACSCanto II flow cytometer (BD Biosciences, USA) and the data were analyzed via FACSDiva software ((BD Biosciences, USA).

2.7. Detection of the differentiated HL-60 cells

Cell detection was conducted using the neutrophil-like HL-60 cells. After 7 days from the addition of DMSO, the differentiated HL-60 cells (dHL-60) were collected in the tube, and combined with an equal volume of 0.1% (v/v) trypan blue solution for 5 min. Cells were placed in a hemocytometer, which counted the dead and viable cells, and then the viability of each cell was ensured. For the detection of the cells, various concentrations of the cells (1 x 10^3, 1 x 10^4, and 1 x 10^5 cells per 100 μL) were incubated with the aptamer-modified electrode in Dulbecco's phosphate buffered saline for 30 min at RT, and then each impedance value was recorded. The detection of HL-60 cells (1 x 10^3, 1 x 10^4, and 1 x 10^5 cells per 100 μL) was also carried out as control.

2.8. Clinical assessment of the aptasensor

To verify the applicability of the developed sensor for the clinical diagnosis of neutrophil-related diseases, the performance of the IL-17RA aptamer-based sensor was tested using patients' samples. This study was approved by the Institute Review Board of Ajou University Hospital, Suwon, Korea. First, 30 mL of venous peripheral bloods from asthma patients were collected into BD Vacutainer® ACD tubes (BD Vacutainer, Franklin Lakes, NJ, USA) and all preparations were carried out within 2 h after the withdrawal. Each blood was layered on a Lymphoprep^{TM} (Axis-Shield, Norway), followed by centrifugation at 2000 rpm for 25 min at 20 °C without braking. The red blood cells (RBCs) and granulocytes were deposited with Hanks' balanced salt solution (HBSS) buffer (Sigma, USA) containing 2% (w/v) dextran (Fisher Scientific, USA) and incubated for 30 min at RT. The neutrophil-rich upper layer was collected and centrifugally washed with HBSS buffer at 300 g for 10 min at 4 °C. Contaminating RBCs were removed by hypotonic lysis. Neutrophils were resuspended in RPMI-1640 medium supplemented with 2% (v/v) heat-inactivated FBS, penicillin (100 U/mL), and streptomycin (50 μg/mL) (all from Gibco, Thermo Fisher Scientific). The cells were stained with trypan blue to determine the viability (>95%), and the purity of neutrophils (>95%) was determined by hematoxylin and eosin staining. The expression of CD68 was evaluated by flow cytometry. Selected neutrophil cells were applied to the aptasensor at a concentration of 1 x 10^5 cells per 100 μL, and EIS analysis was conducted according to the previous experiments as described above.

3. Results and discussion

3.1. Fabrication of the aptasensor for IL-17RA

Prior to the design of the electrochemical sensor, highly purified IL-17RA was prepared through a series of FPLC. As shown in Fig. S1, the final purity of IL-17RA was greater than 95% (approximately 62 kDa), as revealed by the relative band width on an SDS-PAGE gel.

To construct the IL-17RA-specific biosensor, we introduced EIS that was suitable to measure the interaction between probes immobilized on a surface of an electrode and a trace amount of target molecules in a liquid phase. Accumulation of non-conductive materials onto the surface of the electrode caused by the specific interaction disrupts the electron transport between a solution and the electrode, resulting in an increase in the resistance value at the electrode. Based on these resistance values, sensitive detection is achievable. Such an impedimetric sensor can be improved via various modification of the surface of the electrode. The deposition of AuNPs is one of the better techniques for the enhancement of EIS because of high electrical conductivity, biocompatibility, large active surface, and ease of self-assembly (Castaneda et al. 2007; Chen et al. 2011). An electrodeposition method that enables the synthesis and deposition of AuNPs instantaneously is especially fascinating (Lee et al. 2015).

As illustrated in Fig. 1, the aptasensor was gradually fabricated through a series of functionalization steps as described in the experimental section. Each modification process was monitored by CV (Fig. S2). After the electrodeposition of AuNPs, the 5’ thiol-modified IL-17RA aptamer was immobilized onto the surface of the working electrode via gold-thiol interaction. While the electrodeposition of AuNPs made no significant changes in the oxidation and reduction peak currents compared with those of bare electrode, the immobilization of the aptamer caused striking decreases in the peak currents, implying that

the electrode was well modified with the aptamers. The addition of IL-17RA also gave rise to a reduction in the currents, indicating that the IL-17RA aptamer with a very low K_d value (14.00 nM) offered by Aptamer Sciences showed effective binding ability with IL-17RA.

3.2. Optimization of the aptasensor for IL-17RA

When the immobilized aptamer interacts with the target molecule, it is clear that a spacer, which offers adequate space for the binding, could influence the binding extent. Therefore, various concentrations of 6-mercapto-1-hexanol (0, 25, and 50 nM) with 250 nM of thiol-modified IL-17RA aptamer were incubated with the AuNPs-deposited working electrode, followed by the reaction with IL-17RA. Then, the Nyquist plots were obtained and the charge transfer resistance (R_{ct}) was measured using PowerSuite software (ver. 2.55, Princeton Applied Research). R_{ct} signifies the diameters of the semicircles in the Nyquist plots, which indicates the surface resistance of the electrode for transporting the electrons of the $[Fe(CN)_6]^{3-/4-}$ ions. Fig. S3 represents relative ratios of R_{ct} compared to that of only the aptamer-immobilized electrode (0 nM). In most cases, the introduction of spacers makes the interaction between target molecules and DNA probes easy, leading to an increase in the surface resistance. However, the R_{ct} values decreased in proportion to the concentration of the spacer in this work. It seems that an uncommon base in the IL-17RA aptamer, 5-[N-(1-naphthylmethyl)carboxamide]-2'-deoxyuridine (Nap-dU) replacing dT, is a primary factor for this disparity. This substitution provides high binding affinity with target molecules, especially IL-17RA, and superior resistance against degradation by several nucleases (Gupta et al. 2014). Because of the Nap-dU, the structure of the aptamer might be different from general DNA structures, so unusual results could be obtained, but a detailed discussion about the aptamer structure is beyond the scope of this article. As a result, we utilized only the IL-17RA aptamer without any spacer in the fabrication of the sensor.

3.3. Confirmation of the analytical performance of the aptasensor

Using the developed impedimetric sensor, the quantitation of IL-17RA was carried out. To improve reproducibility of the sensor, the working electrodes having similar impedance values were utilized to the detection. Several concentrations of IL-17RA ranging from 10 pg/mL to 10 ng/mL were incubated with the aptamer-immobilized electrode, then, each impedance value was recorded. All measurements were performed in triplicate. As exhibited in Fig. 2A, R_{ct} values increased proportionally to the concentrations of IL-17RA, and the calibration curve showed a good linear relationship (Fig. 2B) between the concentration of IL-17RA and the R_{ct} value with a high square of the correlation coefficient (R^2=0.9922), suggesting that this aptasensor offers great reproducibility. The average coefficient of variations was 6.02%, which is lower than that of ELISA kits (intra-assay coefficient of variations: <10%; inter-assay coefficient of variations: <12%). The designed aptasensor validated a good analytical performance for IL-17RA with a dynamic range of 10 – 10000 pg/mL in a buffer and a detection limit of 2.13 pg/mL (LOD=3s/slope; s: standard deviation of samples), which is lower than those of commercially available ELISA kits (10 pg/mL), indicating that this biosensor is valuable to quantify IL-17RA.

[Fig. 2]

3.4. Demonstration of the selectivity of the biosensor

Specificity is another crucial feature of a target-specific biosensor. Even though the specificity of the IL-17RA aptamer was already validated by the supplier, it is indispensable that the selectivity of the aptamer-immobilized sensor be verified. Therefore, diverse types of proteins (IL-5R, IL-13R, CD166, HSA, BSA, and lysozyme) were applied to the aptasensor

at an identical concentration. On the basis of the R_{ct} value of IL-17RA, the relative ratios of each R_{ct} were presented in Fig. 3A. The R_{ct} values of other proteins were relatively lower than that of IL-17RA, revealing that the fabricated aptasensor is capable of selectively detecting only IL-17RA. In the case of the commercially available ELISA kits, the specificity was demonstrated using more than 46 proteins, resulting in no cross-reactivity with other interfering materials. The designed sensor also exhibited a comparable selectivity to that of ELISA kits. In addition, the aptasensor was tested in a human serum solution. The human serum was diluted 10-fold with PBS (pH 7.4), and then samples were made by supplementing several concentrations of IL-17RA (100 pg/mL, 1 ng/mL, and 10 ng/mL), followed by the measurements using the sensor. Fig. 3B displays the sequential increase of R_{ct} values in accordance with the increase in the concentration of IL-17RA. Fig. 3C also represents a good linear relationship between them (R^2=0.9674), suggesting that the electrochemical aptasensor operates very well in a biochemical environment analogous to blood.

[Fig. 3]

3.5. Application to cell enumeration

[Fig. 4]

It has been widely known that human promyelocytic HL-60 cells can be differentiated to neutrophil-like cells in compliance with a variety of chemical stimuli (Martin et al. 1990). To authenticate the applicability of the aptasensor for cell detection, neutrophil-like cell lines were prepared using DMSO as described in the experimental section. The degree of differentiation was confirmed via FACS using dye-labeled antibodies (CD11b and

CD14). Whereas there was no increase in the ratio of double-positive neutrophil-like cells in the case of native HL-60 cells, DMSO-treated HL-60 cell lines were gradually differentiated to neutrophil-like cells as time passed. After 7 days from the treatment of DMSO, approximately 51 percent of HL-60 cells were successfully differentiated to neutrophil-like cells, implying that the half of DMSO-treated total HL-60 cells are neutrophil-like cells (Fig. 4). Some concentrations of dHL-60 and HL-60 cells (1×10^3, 1×10^4, and 1×10^5 cells per 100 μL of PBS) were applied to the designed aptasensor, and each R_{ct} value was recorded as represented in Fig. 5. In the case of HL-60 cells, there was no significant changes in R_{ct} values in proportional to the cell concentrations (Fig. 5A). Although R_{ct} values consecutively increased according to the number of cells, the linear response to cell concentrations was not observed, insinuating that heterogeneous expression of IL-17RA on the surface of cells interrupts the exact quantification of cells (Fig. 5B). Therefore, the developed electrochemical sensor can be employed not to directly count the cells, but to provide sensitive detection of neutrophils.

[Fig. 5]

3.6. Clinical application of the fabricated aptasensor

As previously described, the neutrophils were selectively separated from the blood of three asthma patients and verified using CD68 antibodies via FACS. A total of three adult patients with asthma were recruited at the Ajou University Hospital, Suwon, South Korea, and informed consent was provided from all subjects. Each neutrophil was incubated with the aptasensor with a concentration of 1×10^5 cells per 100 μL. Though there was a difference in R_{ct} values, the treatment of neutrophils from asthma patients effectively increased the surface resistance of the electrode as illustrated in Fig. 6. It seems that the discrepancy among R_{ct}

values results from diverse factors such as unequal expression of IL-17RA on the neutrophils, the inconsistency of surface components in the neutrophils, and interference by some biomolecules in blood. Nevertheless, the aptasensor showed great responses towards neutrophils, so it has value as a diagnosis tool for neutrophil-mediated innate immune resistance.

[Fig. 6]

4. Conclusion

In this study, a highly sensitive and selective electrochemical aptasensor for IL-17RA, a diagnostic biomarker for several immune diseases, including autoimmune disease, was developed based on EIS. To improve the sensitivity of the sensor, AuNPs were electrodeposited onto the working electrode, and then 5'-thiol modified IL-17RA aptamer was immobilized on the electrode. Each modification step was monitored using CV. The fabricated aptasensor was optimized by varying the concentration of the spacer. The impedimetric detection for IL-17RA was conducted by the measurement of R_{ct} values, and IL-17RA was successfully detected with a detection limit of 2.13 pg/mL in buffer conditions, which is sufficiently lower than that of an ELISA kit. Furthermore, this biosensor distinguished only target IL-17RA among other proteins, and showed a good performance in a solution supplemented with human serum. In addition, the aptasensor could detect the dHL-60 cells that express IL-17RA with fair sensitivity, as well as neutrophils from asthma patients. For the application of this system to clinical devices, we have made an endeavor to overcome various obstacles such as degradation of DNA probes by several nucleases in blood, signal fluctuation due to interfering materials, and ineffective signal transition in a small detection system. Various solutions such as the addition of nuclease inhibitor to the samples, the modification of nucleotides in the aptamer, and the pre-separation of the interfering cells are valuable to improve the aptasensor. It is anticipated that a highly sensitive aptasensor will be readily applicable to the diagnosis and prognosis of IL-17RA-related diseases.

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Figure captions

Fig. 1. Schematic illustration of the detection for IL-17RA. After the electrodeposition of AuNPs, the IL-17RA aptamer was immobilized on the electrode. The concentration of IL-17RA was determined based on the increase in impedance.

Fig. 2. Quantification of IL-17RA using EIS. (A) Nyquist plots for the electrochemical detection of IL-17RA in PBS. The measurements were carried out using various concentrations of the protein ranging from 10 pg/mL to 10 ng/mL. (B) Linear fit for the R_{ct} vs. log [IL-17RA]. All measurements were performed in triplicate, and the error bars were indicated on the curve.

Fig. 3. (A) Selectivity test of the aptasensor. Each protein was applied to the sensor at a concentration of 1 ng/mL, and the relative ratios of R_{ct} to that of IL-17RA were plotted. The error bars indicate the standard deviations of three repeated measurements. (B) Nyquist plots for the electrochemical detection of IL-17RA in human serum-supplemented solution. The measurements were carried out using various concentrations of the protein ranging from 100 pg/mL to 10 ng/mL. (B) Linear fit for the R_{ct} vs. log [IL-17RA]. All measurements were performed in triplicate, and the error bars were indicated on the curve.

Fig. 4. Differentiation of HL-60 cell lines to neutrophil-like cells. Differentiation was confirmed via FACSCanto II flow cytometer using APC-CD11b and PerCP-CD14. The data were analyzed via FACSDiva software.

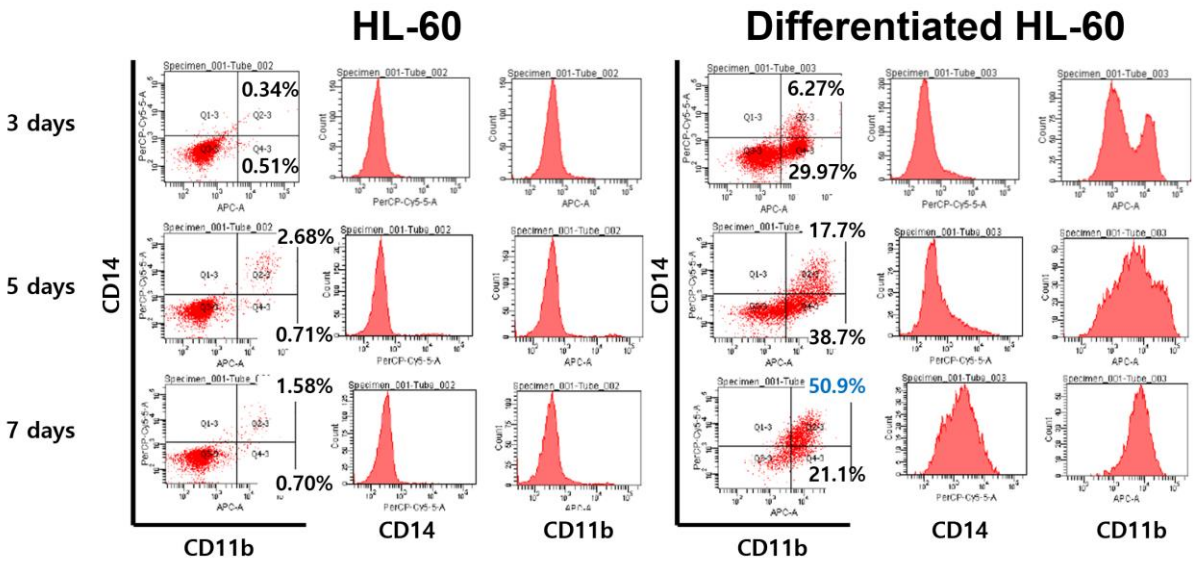
Fig. 5. R_{ct} values for various concentrations of the HL-60 cells (A) and the neutrophil-like

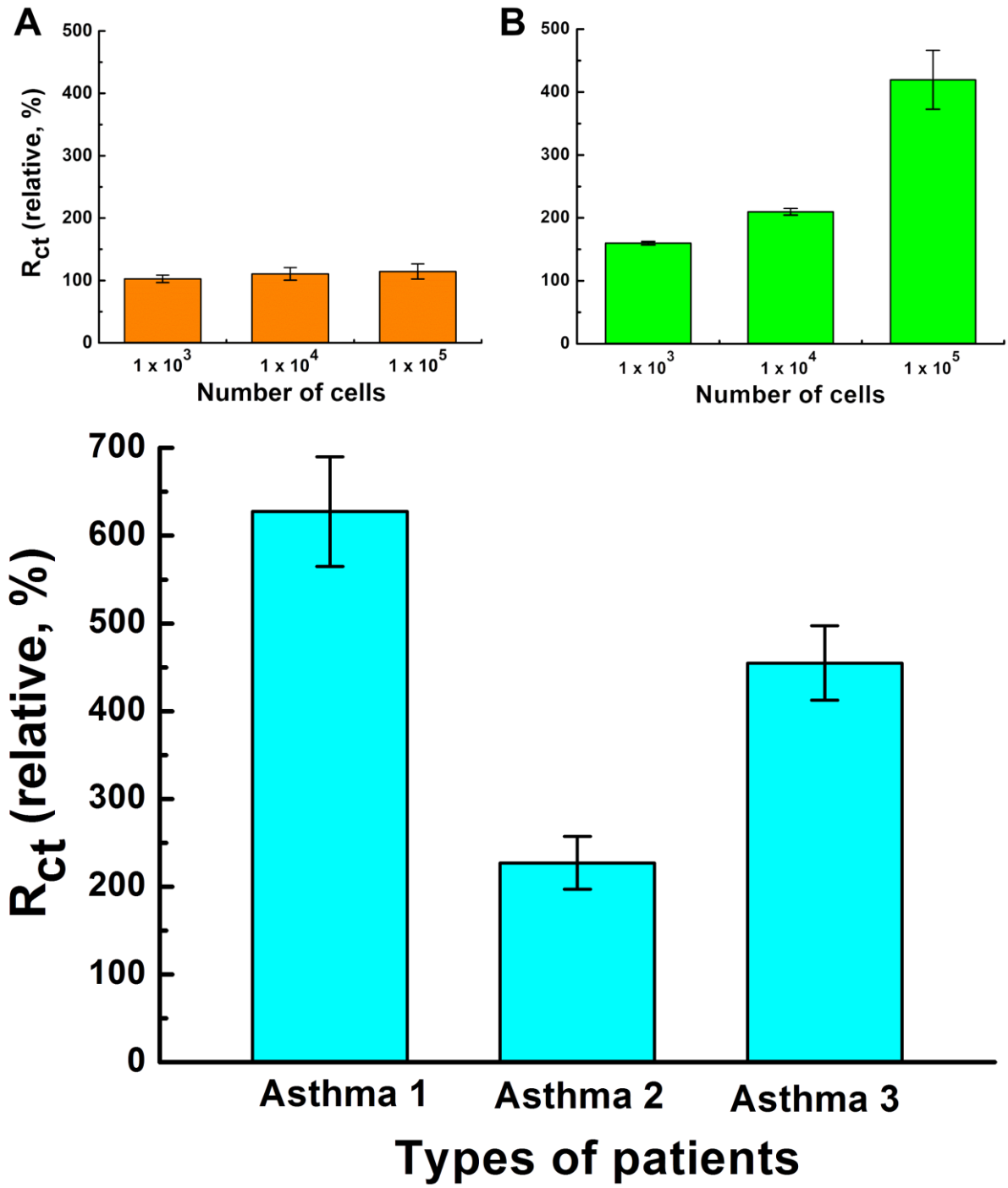
HL-60 cells (B). The error bars represent the standard deviations of three independent experiments.

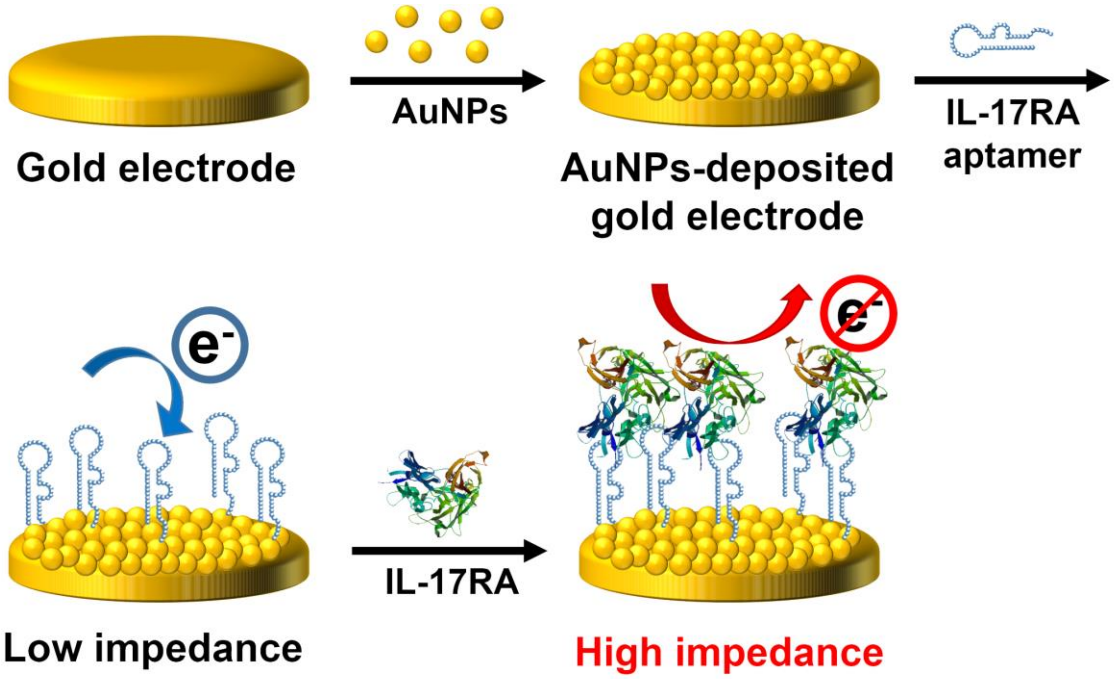
Fig. 6. Clinical application of the fabricated sensor to neutrophils from asthma patients. Neutrophils were employed at a concentration of 1 x 10^5 cells per 100 μL, and all measurements were carried out in triplicate.

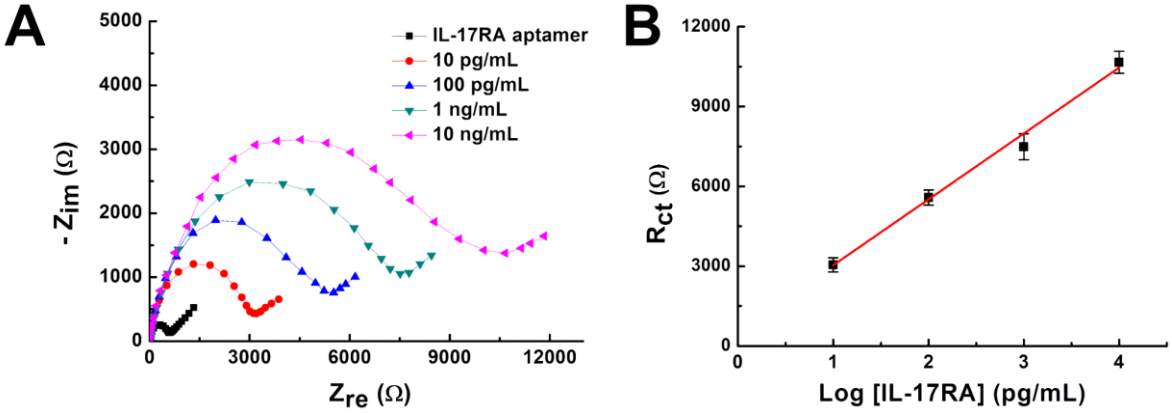
Highlights

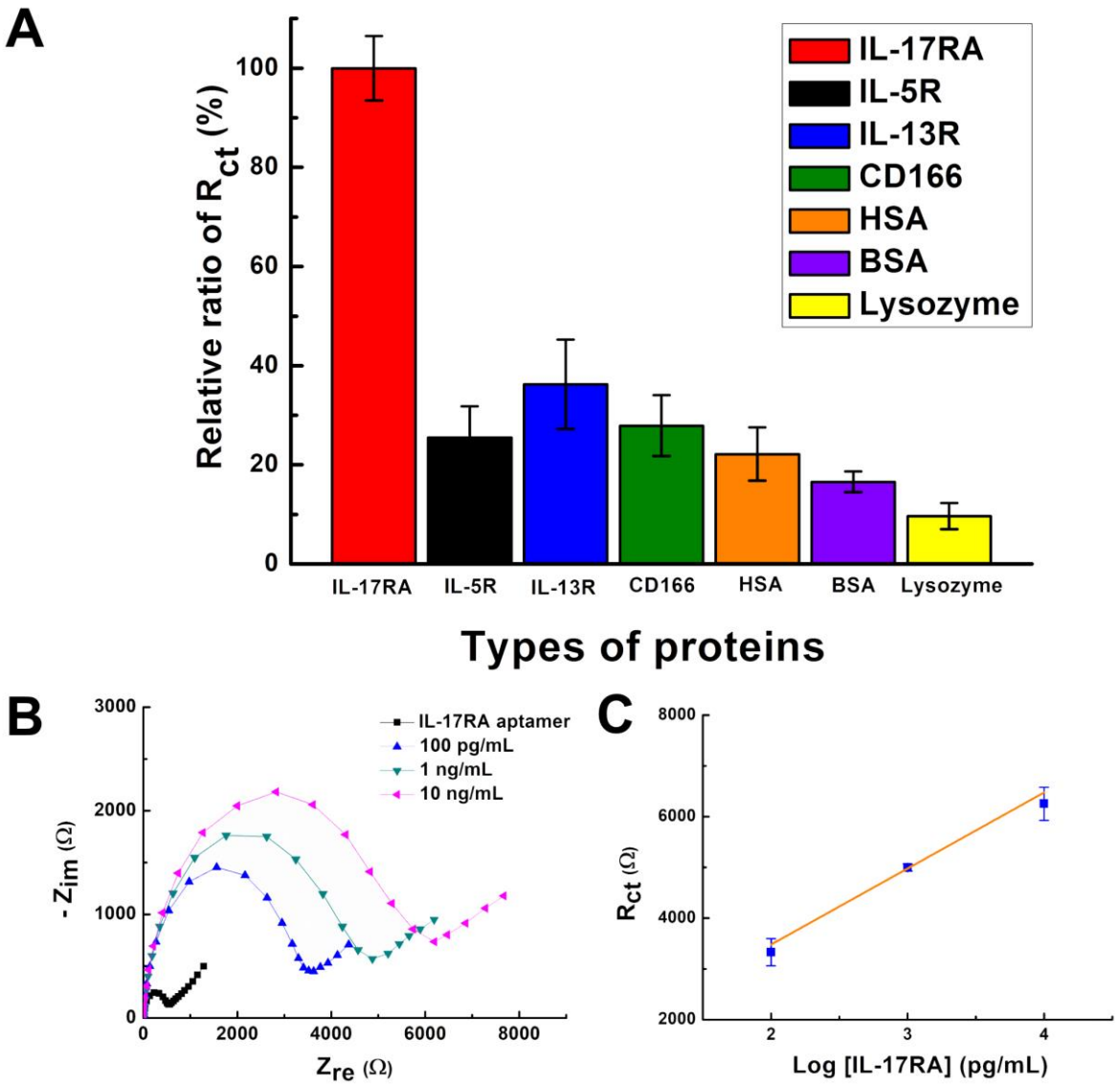
- The design of an aptasensor for IL-17RA.
- A wide dynamic range of 10 – 10000 pg/mL and a detection limit of 2.13 pg/mL.
- Robust sensitivity and selectivity of impedimetric biosensor.
- Successful detection of the differentiated HL-60 cells expressing IL-17RA.
- Great applicability towards clinical samples.










A
Relative ratio of R_{ct} (%)
0
20
40
60
80
100
IL-17RA
IL-5R
IL-13R
CD166
HSA
BSA
Lysozyme
Types of proteins
B
- Z_{im} (Ω)
Z_{re} (Ω)
0
1000
2000
3000
0
2000
4000
6000
8000
IL-17RA aptamer
100 pg/mL
1 ng/mL
10 ng/mL
C
R_{ct} (Ω)
2000
4000
6000
8000
2
3
4
Log [IL-17RA] (pg/mL)