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Three-dimensional interdigitated electrode array as a transducer for label-free biosensors

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ABSTRACT

A new transducer for biosensor applications has been developed based on a three-dimensional interdigitated electrode array (IDEA) with electrode digits separated by an insulating barrier. Binding of molecules to a chemically modified surface of the transducer induces important changes in conductivity between the electrodes. Three-dimensional sensor shows considerable improvement compared with a standard planar IDEA design. The potential of the developed device as a sensor transducer to detect immunochemical and enzymatic reactions, as well as DNA hybridization events is demonstrated. The immunosensor allows direct detection of the antibiotic sulfapyridine and shows the IC_{50} parameter value of 5.6 μg L^{-1} in a buffer. Immunochemical determination occurs under competitive configurations and without the use of any label. Each modified sensor is of a single use. Nevertheless, biochemical reagents can be easily cleaned off the sensor surface for its reuse. Layer-by-layer method of used to deposit polyethyleneimine and glucose oxidase showed that the sensor is also highly effective for detecting single and multilayered molecular assemblies.

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1. Introduction

Impedance biosensors lately gained considerable interest (Katz and Willner, 2003) due to their ability to perform label-free detection (Daniels and Pourmand, 2007) and also due to potentially low cost and ease of miniaturization. Monitoring changes in surface impedance when a target molecule binds to a probe molecule immobilized on a sensor surface permits to detect DNA and protein targets (Berggren et al., 2001).

Like in the widespread enzyme-linked immunosorbent assay (ELISA) technique, most of the reported biosensors rely on some kind of an easily detectable label attached to the target molecule. Labels can be fluorophores, enzymes, metal nanoparticles, magnetic beads, and others. However, the need to obtain molecules with attached labels raises the cost of the analysis.

Impedance biosensors register changes in the electrical properties of the surface (either capacitance or resistance) affected by interactions of a target biomolecule with a probe-functionalized sensor surface and are promising for label-free, real-time, and in situ detection of various analytes. They have been used for immunochemical reactions (Bataillard et al., 1988; Berggren et al., 2001) as well as for direct measurements of DNA hybridization processes (Dharuman et al., 2005; Li et al., 2006). To enhance the sensitivity of the measurements and to miniaturize the final sensor element an impedimetric transducer with two planar interdigitated electrodes, called interdigitated electrode array (IDEA), was introduced (Laureyn et al., 2001; Van Gerwen et al., 1998). This type of a sensor is presented in Fig. 1A.

Certain molecules may be immobilized on the top (Fig. 1B) or in between (Fig. 1C) the electrode digits. These molecules 'recognize' a specific analyte via chemical interactions when exposed to a sample solution. This recognition process eventually ends up directly altering the conductivity and/or permittivity of the electrodes neighbourhood space. By measuring the impedance between the two electrodes, a measure of the recognition process can be established by fitting parameters of the electrical equivalent circuit (Daniels and Pourmand, 2007) presented in Fig. 2A to the spectrum shape. Elements of the equivalent circuit have the following physical significance: R_C—contact resistance of wires, contacts and collector bars; C_G—geometrical capacitance between two

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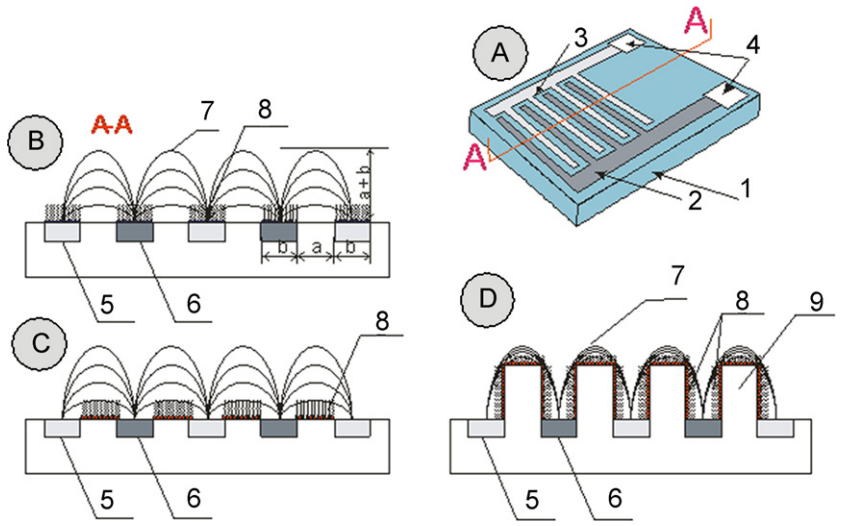

Fig. 1. (A) Planar IDEA device, (B and C) cross-section A–A of the planar IDEA device, and (D) IDEA device with insulating barriers between electrode digits. (1) Insulating substrate, (2 and 3) electrodes collector bars, (4) contact pads, (5 and 6) electrode "digits", (7) electric field lines, (8) immobilized biomolecules, and (9) insulating barrier.

electrodes through the ambient in contact (typically water solution); R_S—electrical resistance of water solution between two electrodes; C_{DL}—double layer capacitance at the electrode/solution interface; R_{CT}—charge transfer resistance of faradaic processes at the electrode surface; W—additional polarisation that may be caused by concentration polarisation (Warburg impedance), roughness of the electrodes surface and/or presence of additional layer on the electrode surface.

Large amount of reported impedance biosensors use $[Fe(CN)_6]^{(3-/4-)}$ as redox marker ions in test solutions monitoring the change in the electron transfer resistance caused by affinity reactions, like hybridization with a complementary DNA (Ito et al., 2007) or immunoreactions with an antigen (Garifallou et al., 2007).

Another parameter that is often used for registering affinity reactions on a sensor surface is a capacitance of a biomolecules layer, C_F, that goes in series with the double layer capacitance, C_{DL} (Berggren et al., 2001; Guiducci et al., 2006; Jiang et al., 2003).

If no redox species are present in the solution or if the electrode/solution interface behaves as a blocked one, the equivalent circuit of an IDEA sensor may be simplified as presented in Fig. 2B (Daniels et al., 2007; Lvovich et al., 2006).

Planar IDEA devices used as capacitance sensors for label-free detection with molecules immobilized on top of the electrodes (8, Fig. 1B) present important limitations. To accomplish the required sensitivity and detectability in this case the immobilized layer covering the conducting electrodes should be perfectly homogenous and should not contain holes, which is hard to achieve.

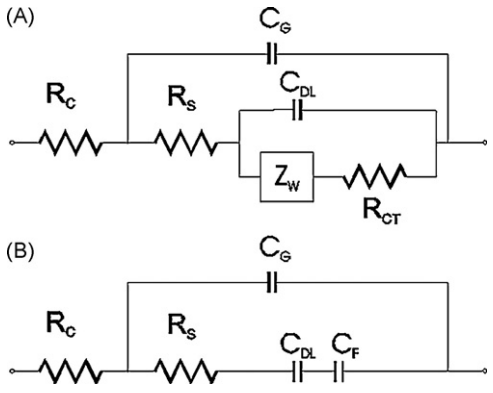

Fig. 2. Equivalent circuit model of an IDEA sensor (A); simplified model in the absence of faradaic processes at the metal/solution interface (B).

When these molecules are immobilized between the pair of electrodes they will considerably affect the sensor impedance only when digits dimensions and interspacing are comparable to the biomolecule length, which is difficult to achieve with conventional microelectronic technology. In the opposite case, the effect of the biomolecules will be negligible and the measured impedance will mainly be determined by the conductivity of the solution and the double layer capacitance at the metal/solution interface. This is due to the fact that parameters of impedimetric sensors based on interdigitated electrodes depend on the electrodes geometry, i.e. dimensions and interspacing (parameters a and b in Fig. 1B). It is generally accepted (Mamishev et al., 2004) that 80% of the total signal is enclosed in a certain region close to the electrode surface. Electric field penetrates within the distance equal to the distance between centres of two adjacent electrode digits (5 and 6, Fig. 1), as shown in Fig. 1B, where the electric field lines are schematically marked. Typical biomolecule length is within the range of 1–100 nm, so the gap between the IDEA digits should be of the same order of magnitude.

Due to the difficulties mentioned above, reported up to now planar impedimetric sensors for direct measurements of biochemical interactions show low detectability in comparison with other conventional methods.

Taking into consideration the distribution of the electric field between adjacent electrodes of an IDEA sensor it seems reasonable to separate the electrodes with an insulating barrier so that under applied electric potential difference the main portion of the current will not go through the surrounding solution but close to the surface of the barrier as it is shown in Fig. 1D. This should permit to enhance the sensitivity of the device for biochemical reactions of biomolecules attached to the surface of the barrier.

The aim of this work was to test the possibilities of a new sensor design in different configurations, namely, with antigens, DNA molecules and enzymes immobilized on its surface.

2. Experimental

2.1. Reagents and immunoreagents

The preparation of the immunoreagents SA2–OVA and As155 is described elsewhere (Adrian et al., submitted). Briefly,

the antigen immobilized on the sensor surfaces is a 5-[4-(amino)phenylsulfonamide]-5-oxopentanoic acid (SA2) covalently coupled to OVA (ovalbumin) and has 18 mol SA2/mol protein. The antibody As155 has been raised in New Zealand White rabbits immunized with 5-[6-(4-amino-benzenesulfonylamino)-pyridin-3-yl]-2-methyl-pentanoic acid (SA1) coupled to HCH (horseshoe crab hemocyanin). 3-(Glycidoxypropyl)trimethoxysilane (GPTS) used to derivatize the transducer surface and other biochemicals were obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO). Sulfapyridine used as standard antibiotic analyte was supplied by Riedel-de Haën. All inorganic reagents used in this work were at least of analytical grade purity. Solutions were prepared using deionized Milli-Q water.

2.1.1. Buffers

Unless otherwise indicated, phosphate-buffered saline (PBS) is 0.01 M phosphate buffer and 0.8% saline solution, pH 7.5. PBST is PBS with 0.05% Tween 20. Borate buffer is 0.2 M boric acid–sodium borate, pH 8.7. Coating buffer is 0.05 M carbonate/bicarbonate buffer, pH 9.6. Citrate buffer is a 0.04 M solution of sodium citrate, pH 5.5. The substrate solution contains 0.01% TMB (3,3′,5,5′-tetramethylbenzidine) and 0.004% H_2O_2 in citrate buffer. The buffer used to make the impedimetric measurements is 400 nM PBS, prepared by diluting PBS with Milli-Q water to obtain a final conductivity of 1.6 $\mu S\,cm^{-1}$.

The hybridization buffer is 10 mM Tris–HCl, 10 mM EDTA and 1 M NaCl, pH 7.2.

2.2. Sensor fabrication

The silicon wafer is oxidized thermally by “wet” oxidation at 950 °C to give a good quality silicon dioxide layer of 2500 nm. In the next steps a 230 nm thick layer of tantalum silicide, which is a highly conductive material, is deposited by magnetron sputtering. The first photolithographic step defines collector bars and digits of two electrodes. The patterning is done by a reactive ion etching technique. This results in a symmetrical interdigitated electrode array with 216 digits of 3 μm width separated by a 3 μm gap between the adjacent electrodes. The aperture (overlapping) between the electrodes digits is 1.4 mm and the total length between the electrodes is 301 mm. To form the contact pads 1 μm of aluminium is deposited and patterned using standard photolithographic and etching steps leaving metal only at extremes of the two collector bars.

The final step is the formation of insulating barriers between the electrode digits. To do this the wafer with formed IDEA devices is covered with a 4 μm thick LPCVD silicon oxide layer. Photolithography is used to define the openings in the oxide layer over the electrodes digits and over contact pads. These zones are opened by deep reactive ion etching (DRIE), which permits to obtain barriers with nearly vertical walls. The barrier height separating adjacent electrodes is equal to the thickness of the deposited oxide layer. The sensor design is presented schematically in Fig. 3I(A) along with optical and electronic microscopy images of the sensor surface and obtained barrier profile (I(B)). After being cut from the wafer the sensors are glued to a PCB substrate and are wire bonded for electrical connections. Contact pads and wires were encapsulated using epoxy resin.

Characterization of sensors was performed by impedance measurements in a 100 Hz to 500 kHz frequency range with a 25 mV (amplitude) voltage excitation using PARSTAT 2263 Advanced Electrochemical System. Z-Plot/Z-View software package (Scribner Associates, Southern Pines, NC, USA) was used for impedance data treatment and an equivalent circuit fitting.

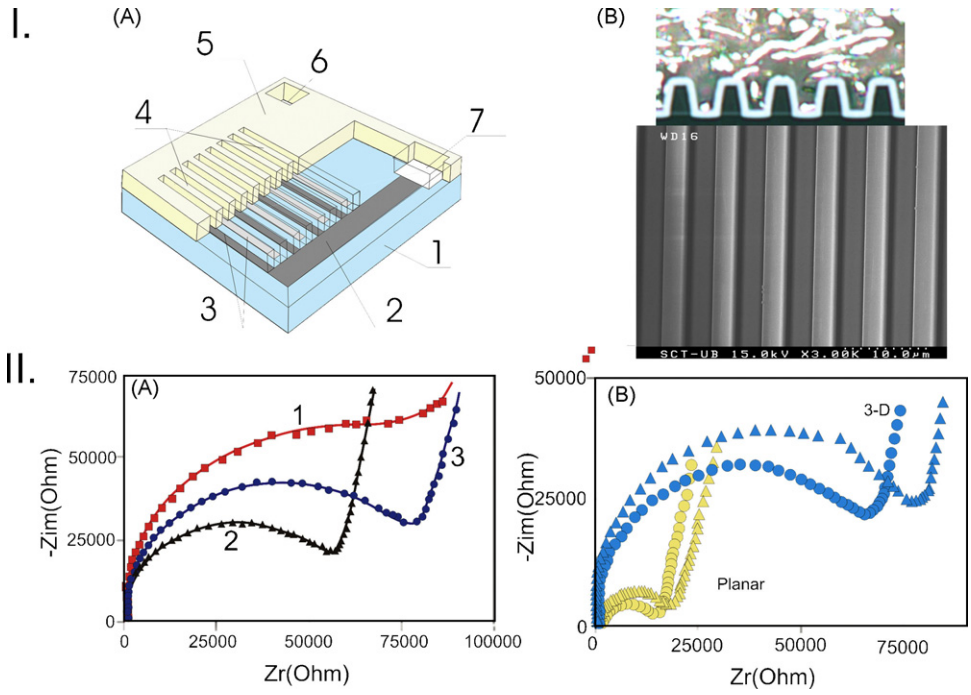

Fig. 3. (I) Design of an IDEA sensor with barriers separating the electrode digits. (A) Schematic presentation of the sensor. (1) Insulating substrate (Si/SiO_2), (2) electrode collector bar, (3) electrode digits, (4) silicon dioxide barriers between the electrode digits, (5) 4 μm thick SiO_2 layer, and (6 and 7) aluminium contact pads. (B) SEM image of the sensor surface and optical microscopy image of the barriers profile. (II) Nyquist plots of impedance spectra corresponding to—A (1) not modified clean sensor, (2) sensor with immobilized antigen, and (3) sensor after immunoreaction with a specific antiserum. Symbols represent the experimental data. Solid curves represent the computer fitting data using the equivalent circuit presented in Fig. 2B of the paper. (B) IDEA sensors modified with 1 $\mu g\,mL^{-1}$ antigen (squares) and after the immunoreaction with 1/1000 As155 (triangles); larger circles correspond to three-dimensional IDEA sensor with insulating barriers; smaller circles correspond to IDEA immunosensor with gold electrodes of the same geometry but of the standard planar design.

The equivalent circuit presented in Fig. 2B was used for spectra fitting. The goodness of the fit may be assessed using the χ^2 and the weighted sum of squares parameters. The first parameter is the square of the standard deviation between the original data and the calculated spectrum and the second is proportional to the average percentage error between the original data points and the calculated values. For all spectra measured in this work the χ^2 parameter was typically smaller than 0.0025 and the weighted sum of squares was in the 0.18–0.30 range. The relative error of the R_S values obtained by fitting was below 0.8%.

2.3. Chemical modification of SiO_2 with silane

Before chemical treatment the IDEA samples were first cleaned with ethanol: Mili-Q water 70:30 and absolute ethanol in an ultrasonic bath for 10 min and dried under nitrogen flow. Followed the samples were immersed in a freshly prepared piranha solution (7:3, v/v, H_2SO_4:H_2O_2) for 1 min and subsequently rinsed thoroughly in absolute ethanol. Immediately afterwards the samples were plunged in a 1 M NaOH aqueous solution at RT for 30 min and rinsed with Milli-Q water and ethanol in order to neutralize the action of the base. After drying them with N_2, samples were immersed into a solution of 3-(glycidoxypropyl)trimethoxysilane in anhydrous ethanol (2.5%, v/v) for 16 h. Finally, the devices were rinsed with absolute ethanol, dried under N_2 flow, and stored in vacuum until utilization.

2.4. Antigen immobilization impedimetric immunosensor measurements

The terminal epoxy group of silane attached to the surface of the SiO_2 readily reacts with amino groups of biomolecules (Lobert et al., 2003; Taylor et al., 2003) thus fixing them on the surface. In this way immobilization of the sulfonamide antigen SA2–OVA (Adrian et al., submitted) on the sensor surface modified with GPTS was performed, The sensors were immersed into a solution of antigen SA2–OVA (0.8 μg mL^{-1} in carbonate buffer). The reaction was performed during 24 h at 25 °C with orbital stirring. The reaction liquid was washed away and sensors were rinsed with PBST buffer (four times, 1000 μL/electrode) and dried with a N_2 stream.

Specific antibody binding was assessed by placing the sensor during 30 min at RT into a vial containing a mixture of the As155 (150 μL, 1/2000 in 10 mM PBS with 0.05% Tween 20) and a solution of the sulfapyridine (SPY, 150 μL, 50,000–0.64 nM in 10 mM PBS with 0.05% Tween 20). The electrodes were washed by dipping them in PBST (5 mL, three times) and in 400 nM PBS (5 mL, one time). After washing the sensor with PBS, the electrodes were dried with N_2 and the impedimetric spectra were recorded in 1 min.

2.5. ELISA method

For the ELISA experiments polystyrene microtiter plates were purchased from Nunc (Maxisorp, Roskilde, DK). Washing steps were performed on a SLY96 PW microplate washer (SLT Labinstruments GmbH, Salzburg, Austria). Absorbance was read on a Spectramax-Plus (Molecular Devices, Sunnyvale, CA). The competitive curves were analyzed with a four-parameter logistic equation using the software SoftmaxPro v4.7 (Molecular Devices) and GraphPad Prism 4 (GraphPad Sofware Inc., San Diego, CA). Unless otherwise indicated, data presented correspond to the average of at least two well replicates.

Microtiter plates were coated with SA2–OVA in a coating buffer (0.625 μg mL^{-1}, 100 μL/well) overnight at 4 °C and covered with adhesive plate sealers. The following day the plates were washed with PBST (four times, 300 μL/well). The sulfapyridine standards (0.0256–50,000 nM) prepared in PBST were added to the microtiter plates (50 μL/well). Simultaneously, a dilution of As155b (1/4000) prepared in the same PBST was added (50 μL/well) to perform the competition step between the analyte and the antiserum. After 30 min of incubation time at RT, the plates were washed again as before, and a solution of anti IgG-HRP (1/6000 in PBST) was added (100 μL/well) and incubated for 30 min more at RT. After a new wash step, the substrate solution was added (100 μL/well). Color development was stopped after 30 min at RT with 4N H_2SO_4 (50 μL/well), and the absorbances were read at 450 nm. The standard curves were fitted to a four-parameter equation according to the following formula:

$$Y = \left[\frac{A - B}{1 - (x/C)D}\right] + B,$$

where A is the maximal absorbance, B is the minimum absorbance, C is the concentration producing 50% of the maximal absorbance, and D is the slope at the inflection point of the sigmoid curve.

2.6. Oligonucleotide immobilization and hybridization

The oligonucleotides were synthesized by solid-phase procedures by Prof. Ramón Eritja (CSIC, Barcelona, Spain) and kindly given to assess performance of the transducer. An oligonucleotide sequence 5′-aminohexyl-CGA GTC ATT GAG TCA TCG AG-3′ with amino group in 5′ position was covalently attached to sensors surface that was previously modified with GPTS by immersing sensors into a solution of oligonucleotide (10 μg mL^{-1}, 300 μL) in carbonate buffer (pH 9.6). After 24 h of reaction at room temperature the interdigitated electrodes were washed with PBST buffer (four times, 1000 μL/electrode) and dried with a stream of nitrogen.

The hybridization was carried out with an oligonucleotide 5′-fluoresceinehexyl-CTC GAT GAC TCA ATG ACT CG-3′ labelled with fluorescein molecule in 5′ position in a solution of hybridization buffer. The electrodes were put in contact with the solution of the oligonucleotide (10 μg mL^{-1}, 300 μL) and incubated for 5 min at room temperature. After this the sensors were washed four times with PBST buffer (1000 μL/electrode) and dried with a N_2 stream. Along with direct sensor measurements hybridization was controlled utilizing fluorescence microscope. Samples were examined under a Leica TCS-SP laser scanning confocal microscope and documented using Leica TCS software.

2.7. Immobilization of glucose oxidase using layer-by-layer method

Cationic polyethyleneimine (PEI) (high molecular weight, water-free from Aldrich) and glucose oxidase (GOx) (151,000 units/g, solid, from Sigma) were used for modification of the IDEA sensors using layer-by-layer (LBL) self assembly technique (Zhao et al., 2006). Before the deposition IDEA sensors were cleaned in piranha solution (H_2SO_4 + H_2O_2, 3:7) and thoroughly rinsed with deionized water.

To perform the deposition the sensors and silicon oxide samples were immersed into the PEI solution (1.5 mg mL^{-1}) for 20 min, rinsed with a stream of water and dried with a nitrogen flow. To deposit the next layer sensors modified with PEI were immersed for 20 min into a solution of GOx (2 mg mL^{-1}), rinsed and dried. This procedure was repeated several times until a desired number of layers was formed. After each deposition step impedance spectra of sensors were taken in 1×10^{-6} M KCl solution.

Effect of the glucose concentration in water solutions on the impedance of the IDEA sensors was tested using devices with the following multilayer films—(PEI–GOx)$_2$PEI, (PEI–GOx)$_3$PEI and (PEI–GOx)$_4$PEI. Five calibration solutions of glucose (D-(+)-glucose

from Sigma) were prepared in the physiologically important concentration range between 1×10^{-3} and 1×10^{-2} M with the background of 1×10^{-6} M KCl. Impedance spectra were measured after one minute of a sensor's contact with a solution. After each measurement sensors were washed with deionized water and dried with nitrogen.

3. Results and discussion

To test the designed sensor response to biomolecular interactions at its surface it was chemically modified with the sulfonamide antigen SA2–OVA. Fig. 3II(A) shows the measured impedance spectra of the initial clean sensor, sensor with immobilized antigen and the same sensor after the reaction with specific antiserum.

The semicircle at high frequencies is associated with the parallel combination of the electrical resistance between two electrodes, R_S, and the geometrical capacitance C_G of the IDEA device. The capacitance C_G remains constant and equal to 92 ± 3 pF in all the experiments. The straight line at low frequencies appears due to the double layer capacitance, C_{DL}, at the metal electrode/water solution interface with typical values of 10 nF.

As follows from experimental data presented in Fig. 3II(A) immobilization of the antigen on the surface of the sensor provokes increase in conductivity between two electrodes. Immunochemical reaction with the specific antiserum results in higher R_S resistance values. As all the measurements were carried out in the same solution at constant low conductivity, these changes are attributed to the alterations of the properties of the surface layer of the silicon dioxide barrier separating adjacent electrode digits chemically modified with biomolecules.

To test the benefits of the proposed three-dimensional sensor design IDEA sensors with gold electrodes of the same geometry but of the standard planar design (see Fig. 1A) were fabricated. Due to the specificity of the chemical modification processes antigen immobilization on these devices occurs over the silicon dioxide in the gap between the gold electrode digits, as it is presented in Fig. 1C.

As follows from Fig. 3II(B) immunochemical reaction on a sensor surface in case of the IDEA immunosensor based in the standard planar design produces minor changes in the impedance spectra. In comparison to this, response of the three-dimensional sensor shows considerable improvement. This is due to the fact that the main portion of the probing current goes not through the surrounding solution, as in case of a planar sensor, but close to the surface of the barrier with biomolecules attached to its surface.

Analysis of obtained spectra showed that the only component of the equivalent circuit presented in Fig. 2B that was changing significantly as a consequence of the immunoreaction was the resistance, R_S, between two electrodes. Thus, the difference of the R_S values of the antigen-modified sensor determined before and after the reaction with the antibody was taken as the measure of the response, Ω.

Fig. 4 shows the response of the immunosensors to the changes in the sulfapyridine concentration in standard solutions. For comparison results of a standard ELISA performed with the same immunoreagents are presented.

For both methods the calibration curves were fitted to a four-parameter equation according to the formula presented in the experimental section Parameters obtained by fitting both curves are presented in Table 1.

The sensor detectability was estimated with the help of the IC_{50} parameter (analyte concentration causing 50% inhibition of the antibody binding). As follows from Table 1, IC_{50} values for direct sensor measurements and for ELISA method are of the same order of magnitude. The standard deviation of the IC_{50} was

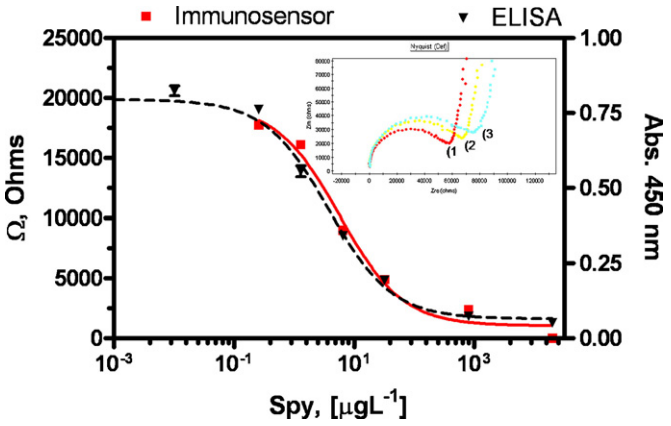

Fig. 4. Response of the IDEA sensor with 4 μm barriers modified with antigen SA2–OVA to sulfapyridine (dashed line). For comparison results obtained with the standard competitive ELISA (solid line). In the inset: impedance spectra of 50 mM (1), 16 nM (2) and 0.064 nM of SPY (3).

0.32, calculated from the four-parameter equation. The slope is the parameter that characterizes the sensitivity and the accuracy of the method. For both methods the same value was obtained. This, along with a high Max/Min ratio, guarantees sufficiently wide analytical working range. Presented results demonstrate that the developed IDEA immunosensor provides sufficient detectability to accomplish with the international regulations regarding sulfonamide residues in food samples. Moreover, the analysis is much faster and the procedure is easier to perform than a traditional ELISA. At present, impedimetric measurements can be taken in just 30 min, but this time can be even lower when a semi-automatic system with immunosensor chips will be developed.

It should be mentioned that each modified sensor is of a single use. Nevertheless, biochemical reagents can be easily cleaned off the sensor surface for its reuse.

Another wide area of biosensors application is DNA determination. Hybridization of DNA constitutes the base for most of bioanalytical tools and for this reason we decided to assess response of our sensor versus this exceptional biorecognition system. Here, once again, direct measurements of reactions between DNA molecules may help to make conventional DNA analysis much cheaper. To test the possibility of the designed IDEA device to detect DNA interactions the following experiments were performed.

Immobilization of the oligonucleotide on the sensor surface using procedure listed above resulted in a decrease of the measured resistance, R_S, as presented in Table 2. Hybridization with the complementary oligonucleotide produced a further decrease in the resistance down to 42.4 kΩ. It should be noted that experiments were performed on three sensors and the relative error of the determined R_S values was less than 0.8%. Hybridization of the oligonucleotides was also confirmed by fluorescence microscopy visualizing the presence of fluorescein on the surface of sensors subjected to the reaction.

Obtained so far results show that the developed transducer is very sensitive to what is happening on the surface of the barriers separating adjacent digits of the electrode array. Taking into consideration that only alterations occurring in a thin layer composed

Table 1
Parameters obtained from calibration with sulfapyridine using direct measurement with the immunosensor and the standard competitive ELISA

	Max	Min	Max/Min	IC_{50} (μg L^{-1})	Slope	R^2
Sensor	19488	1038	18.8	5.6	−0.82	0.99
ELISA	0.79	0.06	13.2	3.72	−0.82	0.99

Table 2
R_S values obtained by fitting the impedance spectra of IDEA devices modified with different layers

Modification		R_S (kΩ)
Immunosensor		
Clean		104.0
Antigen coated		63.83
Antiserum		81.01
DNA sensor		
Clean		95.8
Single strand		52.97
Double strand		44.13
Number of layers		R_S (kΩ)
PEI	GOx	
LBL polyelectrolyte deposition		
0	0	18.04
1	0	6.85
1	1	22.15
2	1	10.27
2	2	27.23
3	2	9.56
3	3	14.34
4	3	9.67
4	4	8.23
5	4	5.08

Reproducibility of the presented data falls within 1%.

by biomolecules at the sensor surface and the adjacent water layer are registered we may assume that changes in the net charge at the surface provoke changes in surface conductivity.

To test this hypothesis we have used a well studied model system of electrostatically assembled monolayers of polyions deposited using layer-by-layer method (Zhao et al., 2006). In our experiments polyethyleneimine was used as a polycation and glucose oxidase (GOx) as a polyanion. Colloid chemistry experiments show that PEI which bears positive charge adsorbs strongly on silicon dioxide increasing to a large extent the surface conductivity of thus modified SiO_2 (Gu and Li, 2000; Sidorova et al., 1993). GOx is negatively charged and behaves as a polyanion interacting with the PEI layers in a water solution (Lvov et al., 1995; Onda et al., 1996, 1999). These two compounds being deposited by LBL method form stable multilayers within which GOx preserves its enzymatic activity (Lvov et al., 1995; Onda et al., 1999). In our experiment we have tested how deposition of these layers affects the impedance of the sensor. In total nine layers were deposited starting with PEI.

As follows from Table 2 deposition of just one layer of PEI notably affects the resistance R_S decreasing its absolute value. In contrast to this, deposition of GOx layer over the PEI layer results in considerable increase of the resistance.

These resistance decrease/increase cycles were registered after each step of PEI/GOx deposition, as reveals Table 2, for the first six layers. Decrease of resistance after deposition of PEI layers may be associated with a high positive charge density within this film which leads to the increase of surface conductivity. Subsequent deposition of the GOx polyanion compensates the charges introduced by PEI. Starting from the seventh layer this effect diminishes probably due to a high intrinsic conductivity of the multilayer film that produces a shunting effect.

Obtained devices with $(PEI + GOx)_2 + PEI$, $(PEI + GOx)_3 + PEI$, $(PEI + GOx)_4 + PEI$ multilayers were tested in solutions of D-(+)-glucose. All devices show a linear increase of the resistance R_S with the increase of the glucose concentration in the physiologically significant glucose concentration range (0.01–0.001 mol/L). The sensitivity depends on the number of the deposited PEI–GOx layers. For 5, 7 and 9 layers the sensitivity was found to be 269.2, 146.5, and 107.6 kΩ/mol, respectively. Taking into consideration that the resistance increases with the concentration of the substrate in the solution we may assume that the device most probably responds to the formation of an intermediate complex between the enzyme and the substrate and not to the formation of the enzymatic reaction ionic products that should increase the conductivity.

4. Conclusions

A new design of an impedimetric transducer based on an interdigitated electrode array is presented. Due to the presence of insulating barriers that separate the adjacent digits of the electrodes the main portion of the probing electrical current goes close to the surface of the barrier. Chemical modification of the barrier surface with biomolecules (antigens, DNA, enzymes) permits to realise direct label-free detection of subsequent analytes in solution. The functional mechanism of the device is based on registration of changes in conductivity at the surface of the barrier provoked by biochemical reactions of immobilized biomolecules. Presented work is limited to tests of possibilities of a new transducer; further work is required to obtain fully functional biosensors. Nevertheless, obtained results show that the studied three-dimensional interdigitated electrode array is a highly sensitive transducer that can be used to develop biosensors of various types for direct label-free measurements.

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