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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.

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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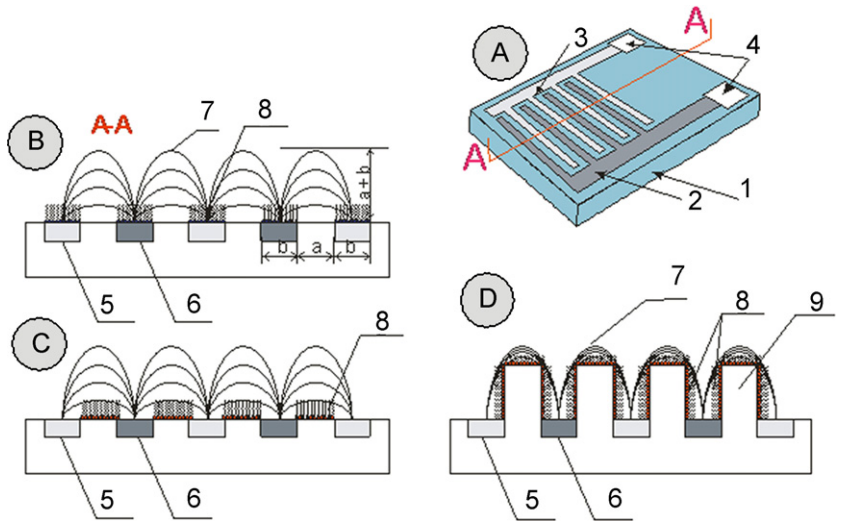

Fig. 1. (A) Planar IDEA device, (B and C) cross-section A–A of the planar IDEA device, and (D) IDEA device with insulating barriers between electrode digits. (1) Insulating substrate, (2 and 3) electrodes collector bars, (4) contact pads, (5 and 6) electrode "digits", (7) electric field lines, (8) immobilized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

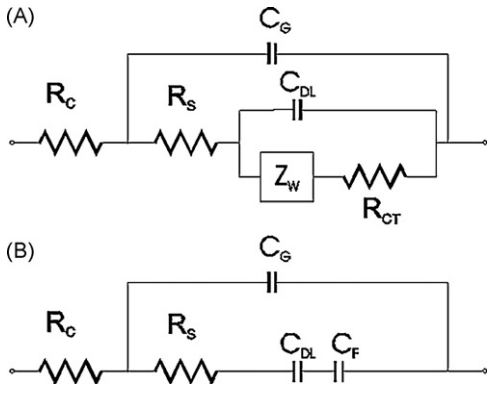

Fig. 2. Equivalent circuit model of an IDEA sensor (A); simplified model in the absence of faradaic processes at the metal/solution interface (B).

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,