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Fabrication and package of ISFET biosensor for micro volume analysis with the use of direct ink writing approach

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ABSTRACT

The present paper suggests a universal foundation for fabrication of biosensor microsystems based on ion-sensitive field effect transistor (ISFET). Combination of complementary metal-oxide-semiconductor (CMOS)-technological solutions and direct ink writing (DIW) technology for creation of the sealed microfluidic system able to deliver the reagents to sensitive ISFET surface is the key feature of the work. The ISFET plays role of a signal transducer with sensitive surface that can be modified with different receptive layers consisting of enzymes, or aptamers, or antibodies. DIW method is used as variation of sacrificial layer technique. 3D scaffold formed of viscoelastic ink is used as the sacrificial layer; epoxy resin works as sealant. Enzymatic biosensors for the determination of c and organophosphate pesticides were developed as a proof of concept. Limit of detection (LOD) for trinitrotoluene (TNT) and paraoxon molecules was estimated as 5 ppb and 30 ppb respectively.

1. Introduction

Use of an ion-sensitive field-effect transistor (ISFET) as a sensor and signal transducer in a chemical analytical system was initially proposed by Bergveld [1]. ISFET is a derivative of MOS (metal-oxide-semiconductor) field effect transistor where analyzed solution is used as a gate instead of metal, with the reference electrode contacting with the gate. Change of surface potential on the phase boundary between the solution and gate insulator during chemical reactions or adsorption of particles on the gate surface produces change of the channel current. The possibility of modifying the gate dielectric surface with enzymes [2], antibodies or aptamers [3,4] turns ISFET into universal platform for different applications.

Nowadays field-effect transistors are widely used in the development of new methods of diagnosis, and the interest of their use as sensing elements for chemical and biochemical systems is increasing every year [5]. Compared with existing diagnostic methods FETs have several advantages. First, the electrochemical conversion of signal allows direct detection of target compound, this avoids an extra step associated with insertion of labels. Another important advantage is possibility of integration of output signal with signal processing system, the automation and miniaturization of the entire system. The miniaturization of the analysis system, in turn, opens up a number of additional features. Reduction of reaction volume allows both to reduce the amount of reagents consumed, which impact on the final cost, and to extract a larger amount of chemical information provided by the sample, which is important in situations where the initial sample volume is limited.

Practical interest in the use of nanostructured transistors is the ability to direct detection of different biomarkers by immunochemical reactions [6,7]. Besides field-effect transistors are promising platform for investigation of nucleic acid interaction in order to for detection of pathogens, drugs screening and genetic diseases [8]. Thus, the direct detection of short oligonucleotides is shown in [9]. At that moment a lot of biosensors based on ISFET and enzymes were developed, allowing to determine the urea [10], creatinine [11], glucose [12], acetylcholine [13], triglycerides [14], blood protein concentration [15] Thus, practical and scientific interest for use of ISFET is great and fabrication of new biosensors will expand the area of ISFET-sensor application.

ISFET can be manufactured following CMOS technology and integrated with single-chip signal processing circuitry, leading to new possibilities of creation of smaller system capable of label-free independent and parallel micro-volume analyses. However, ISFET packaging is a technological challenge for mass fabrication of a ready-to-use biosensor analytical device. Since ISFET uses liquid as a gate, its packaging requires additional finishing operations, making the assembly different from that of standard IC (integrated circuit) and MEMS (microelectromechanical systems). In this case, packaging process implies creation of a miniature fluid interface used to supply reaction

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media to the sensors, as well as sealing unit that is necessary to protect assembled chip and support electrical contacts. It is highly desirable that packaging technology permits to create an assembled chip with an integrated signal processing system comprising a microfluidic system (MFS) that provides maximum sensitivity of the biosensor, and at the same time protecting the microelectronic device against corrosion. It's necessary to notice that the cost of packaging can frequently exceed that of producing the integrated chip [16]. Hence, development of a simple and economical method of ISFET packaging is an important challenge for practical science.

Generally, materials used to create the MFC on ISFET are: polydimethylsiloxane (PDMS) [17], polymethylmetacrylate (PMMA) [18], glass [19], and silicon [20], of which the most commonly used PDMS [21,22] and PMMA [23]. PDMS and PMMA are highly permeable to low-molecular gases (oxygen, nitrogen) as well as humidity [24], which makes them unsuitable for the packaging of microcircuits, since they would not protect their contacts from corrosion. Making MFS of glass or silicon, however, would be much more technically complicated and expensive, comparing to polymer chips. Unwanted immobilization of receptors on microchannel walls during chemical functionalization of ISFET's gate insulator creates further problems, since it leads to the creation of parasitic surface which causes an impairment of the sensor parameters [25].

Direct ink writing (DIW) approach [26] for formation of MFS doesn't have aforementioned drawbacks. Main advantages of DIW approach are:

- universality; it is possible to use different inks and sealing polymers, choosing these reagents according to scientific and practical tasks;
- MFS fabrication is carried out under ambient conditions (room temperature and atmospheric pressure) in the presence of moisture and oxygen, so without requiring special conditions;
- speed, automation and autonomy of the process, as a consequence of the ability for quick transition to large-scale production.

In this paper, we propose a simple method of fabrication and packaging of ISFET biosensor that allows ISFET chips to be manufactured following standard CMOS procedure with further sealing the package with formation of MFS using DIW and epoxy casting. This approach can be easily realized and is used for the first time in combination with ISFET. As a proof of concept, this method was followed to manufacture biosensors for fast detection of trinitrotoluene and paraoxon with use of corresponding enzymes immobilized on the surface of gate insulator.

2. Materials and methods

2.1. Materials

Recombinant *B. diminuta* phosphotriesterase was obtained from MyBioSource, MBS1173203, (California, USA). Recombinant *E. coli* K12 NTR, phosphoric acid solution, petroleum jelly, (3-aminopropyl) triethoxysilane (APTES) and (3-mercaptopropyl)trimethoxysilane (MPTES) solutions, 3-maleimidobenzoic acid N-hydroxysuccinimide ester (MBSE), mineral oil, glutaric aldehyde, $NaHCO_3$, ethyl paraoxon, ethyl parathion, methyl parathion, p-nitrophenol were obtained from Sigma Aldrich (USA). Glutaric dialdehyde was purchased from Merck (USA). Epoxy resin HT2 and its hardener were obtained from PoxySystems (Germany). Methanol, ethanol, and hexane were purchased from Khimmed (Russia). Microcrystalline wax SP-19 was obtained from Strahl and Pitsch (USA).

2.2. ISFET fabrication

P-type silicon-on-insulator (SOI) wafers with 0.18 μm thick 12–22 Ω cm resistivity Si SOI layer and 0.38 μm thick buried oxide layer were the base for fabrication of IC chip with ISFET. N-type ISFETs were manufactured according to 1.2 μm CMOS technology by Scientific and Manufacture Complex "Technological center," Russia. ISFETs were formed in the silicon active layer thinned to 50–70 nm by oxidation and following oxide wet etching. Structures similar to FD (Fully Depleted) MOSFET [27], but without poly-silicon gates, and with 100×100 μm channel were obtained. At the final stage of production, the passivation layer (PECVD Si_3N_4+SiO_2) removal above the FD ISFETs' channel region was performed by plasma chemical etching of Si_3N_4 (Ionbeam-ICP CVD (Oxford Instruments, UK) and wet etching of SiO_2 in HF solution. Pt wire was used as a reference electrode with unpackaged IC.

2.3. ISFET packaging

ISFET packaging included a number of operations, such as cutting of wafer into separate chips using DAD 3350 disc cutter (Disco Corporation, Japan); attaching of chips to the package base with K-400 glue; aluminum wire (diameter of 27 μm) bonding on Delvotec 6400 ultrasonic welding machine (F & K Delvotec); integration of reference electrode (bonding of Au wire with the diameter of 30 μm); and chipping of the packaging lead-frame.

2.4. Formation of microfluidic system

Microfluidic system (MFS) was formed on IC surface using the direct ink writing (DIW) method with sacrificial layer technique.

The sacrificial layer was deposited onto the surface of IC using the water-repellent paraffin ink with Ultimus V pneumatic dispenser (Nordson EFD, Germany) and JR2303 positioning control system (Janome, Japan) as a 3D pattern, the configuration of which coincided with the one of the future MFS.

After that, the packaged chip was poured with epoxy resin. The epoxy material being jellied, the sacrificial layer was etched by heating MFS to 100 °C in the mineral oil, vacuum sucking of the molten ink, and further washing of the mineral oil residues with n-hexane. As a result, a microchannel with two inlets to bring the sample to the sensitive element was formed in the bulk of epoxy polymer.

2.5. Immobilization of the enzyme on silicon oxide surface

Modification of ISFET surface (enzyme modification) was performed on the packaged chip with ready-made MFS. Modification of ISFET sensitive surface implied covalent immobilization of enzymes (nitroreductase (NTR, EC 1.5.1.34) and phosphotriesterase (PTE, EC 3.1.8.1)) on the gate insulator surface which was silicon dioxide. Enzyme immobilization included three sequential stages: incubation with 3% APTES solution in methanol (for 1 h at 22 °C); incubation with 5% glutaric dialdehyde solution in PBS (pH 7.4, for 4 h at 22 °C). Incubation with PTE solution (22 μg/ml) and NTR solution (50 μg/ml) was carried out for 17 h at 1 °C. Chip was thoroughly rinsed between the stages.

2.6. Study of MFS with the immobilized enzyme

Reagent solutions were pumped through the MFS channel with two 2.5 ml syringe pumps. The syringes were mounted into neMESYS syringe pump station (Cetoni GmbH, Germany), which allowed the continuous reagent solutions delivery suitable for switching of the flow from one pump to the other. The first pump was filled in with buffer solution for phosphotriesterase (1 mM $NaHCO_3$, pH 9.0) or nitroreductase (50 mM Na_2HPO_4, pH 7.4); the second pump was filled in with paraoxon solution or trinitrotoluene explosive in the corresponding buffers. The flow velocity was 1 μl/s.

To study ISFET response, the corresponding buffer solution was pumped through the finished system within ~600 s to regulate the