SILICON-BASED GOLD TRANSDUCERS FOR DNA BIOSENSORS

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Abstract: Silicon-based chips with vacuum deposited gold electrode were tested as transducers for the development of DNA-modified biosensors. It was found that these structures are superior over commercially available transducers, mainly due to perfectly smooth surface of gold working electrode. This was confirmed with microscopic and electrochemical experiments. Obtained transducers were modified with oligonucleotide self-assembled monolayer. These sensors were shown to detect chosen DNA sequence with the employment of methylene blue as a redox marker. The same sensors were used to determine UO_2^{2+} cation, however these efforts were unsuccessful.

1 INTRODUCTION

The still-growing number of data which can be used in healthcare, powers the continuing research over the development of new, accurate and cheap methods for the analysis of biological compounds (Luong, Male, Glennon, 2008; Andreescu, Sadik, 2004). The concerns over analysis costs are also very important, as the majority of the clinical analysis are performed in great numbers every day. Various analytical techniques have been used in clinical analysis, each of them having its strengths and drawbacks. The ease of miniaturization, reduction of reagent consumption as well as high sensitivity, reproducibility and accurate analysis are the reasons for high interest in electrochemistry (Wang, 2000). However, to take advantage of all these benefits, the appropriately constructed and mass produced transducers, with well defined surface, allowing for an easy and reproducible creation of the (bio)recognition layer, are required.

Apart from the proper transducer, receptor layer is extremely important for sensor performance. Short oligonucleotides, known as aptamers, have recently gained attention as promising receptors for the construction of novel biosensors. The nucleotides sequence of the DNA strand may be the indicator of several health-important problems, blood contamination with pathogenic bacteria or genetically modified food, thus DNA sequence is one of the most interesting target for aptasensors (Hianik, Wang, 2009). However, the detection of various small biomolecules, as well as inorganic ions can be performed by sensors modified with oligonucleotides.

Another important component, typically present in electrochemical sensors, is a redox marker. In DNA sensors, markers are usually responsible for detection of hybridization or interaction of an aptamer with analyte. There are three possible modes for binding of a marker with DNA, including electrostatic, groove and intercalative binding (Erdem, Ozsoz, 2002). Among compounds employed as electrochemical DNA markers, methylene blue is probably most popular, however the design and application of new redox markers is still an important research topic.

In this work, a holistic approach to the design and fabrication of various aptasensors is presented. The usefulness of the silicon-based transducers with vacuum deposited gold for the construction of such sensors is evaluated. Various geometries of

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transducers were developed and tested, including back-side contact chips and dipstick three-electrode structures. The performance of these transducers was compared with typical gold disc electrodes and commercially available three-electrode structures. The modification of silicon-based chips with oligonucleotide layer, based on self-assembling process, was also performed. Obtained sensors were used for detection of chosen DNA sequence. Moreover, the efforts to determine UO_2^{2+} cation concentration using similar biosensors, will be described.

2 EXPERIMENTAL

2.1 Apparatus

Electrochemical measurements were conducted with a CHI 660A electrochemical workstation (CH Instruments, USA). Voltammetric experiments were carried out with a three-electrode system. If applied, external auxiliary electrode was a gold wire, while Ag/AgCl/1.0 M KCl was used as an external reference electrode (Mineral, Poland). The sample solutions were deoxygenated with argon for approximately 15 minutes prior to data acquisition and were blanketed under an argon atmosphere during the entire experimental period. The square wave voltammetry (SWV) was conducted at a pulse amplitude of 25 mV, step of 1 mV and frequency of 25 Hz. The potentials of chronoamperometry (CA) assay were changed from the initial E = -200 mV to E = 900 mV and it was kept constant for 0.5 sec. The surface pictures were taken by the TM-1000 electron microscope (HITACHI, Japan) and the stereomicroscope SZX10 (Olympus, Japan) coupled with CCD color camera ColorView (Olympus, Japan).

As working electrodes, silicon-based gold transducers with back-side contact (BSC) (Ziolkowski et al., 2010) and vacuum deposited gold, as well as 3-electrode structures (TES) based on silicone wafer, with vacuum deposited gold working electrode (Institute of Electron Technology, Poland), were used (Figure 1). For fabrication of these transducers, a planar CMOS-compatible process was used. For comparison, typical gold disc electrode (Mineral, Poland) (GDE) and commercially available transducers, based on ceramic support, with screen printed gold (SPG) working electrode (BVT Technologies, Czech Republic), were also tested.

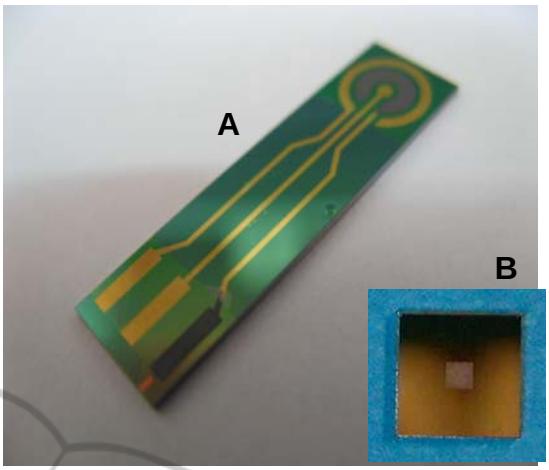

Figure 1: Silicon-based transducers used in this work: A – three-electrode structure (TES) (dimensions 7x26 mm); B – back-side contact structures (dimensions 5x5 mm).

2.2 Reagents

Analytical-grade $K_4Fe(CN)_6$, $K_3Fe(CN)_6$, KCl, K_2HPO_4, KH_2PO_4, NaCl, NaOH, HCl, Tris-HCl, methylene blue, $UO_2(NO_3)_2$ and ascorbic acid were purchased from Aldrich Chemicals. Absolute ethanol was purchased from POCh, Poland. All reagents were used without further purification. All solutions were prepared using Milli-Q water. Milli-Q water and all buffers were sterilized using an autoclave. The 20-mer deoxyoligonucleotides were purchased from Genomed Sp. z o. o., Poland. The base sequences were as follows:

- thiolated DNA probe: 5'-SH-(CH2)6-TCCAACACTCCGAGACGGGG-3'
- complementary DNA target 5'-CCCCGTCTCGGAGTGTTGGA-3'.

All oligonucleotide stock solutions were prepared with 10mM Tris–HCl, (pH 7.5) and stored in a −20 ºC freezer before use.

2.3 Solutions

The following solutions were prepared: piranha solution (H_2O_2:H_2SO_4; 3:1); 0.01 M solution of $K_4Fe(CN)_6$ and $K_3Fe(CN)_6$ in 0.1M KCl; immobilization buffer solution containing 1 M KH_2PO_4 (pH 4.5); hybridization buffer solution containing 10 mM Tris–HCl and 1 M NaCl (pH 7.0). Buffer solution for electrochemical measurements was composed of 0.05M K_2HPO_4/KH_2PO_4 and 0.3 M NaCl (pH 7.0). The 10 µM methylene blue stock solution was prepared in the electrochemical buffer. The pH was adjusted with either NaOH or HCl solution.

2.4 Methods

The pictures of the surfaces were taken according to the microscope producers' instructions (HITACHI, Olympus, Japan).

The real surface area was calculated using the Cottrell equation with the data from chronoamperometric experiments.

To prepare DNA-modified sensors, transducers were cleaned with piranha solution and electrochemically prepared by cycling the potential scan between –0.6 and 1.8V at the scan rate of 0.1V s^{-1} in electrochemical buffer. Subsequently, drop of 4 μM thiolated ssDNA probe in immobilization buffer was placed on the electrode surface and the whole chip was placed in the Petri dish lined with a blotting paper soaked with immobilization buffer. The immobilization was carried on for 90 min at room temperature. This recognition interface was then electrochemically examined with methylene blue solution (Kelley et al., 1997). After analysis, the methylene blue was washed away and the modified gold electrode was exposed to sample solution. After that time, chips were rinsed with phosphate buffer and again the electrochemical examination in methylene blue solution was conducted.

3 RESULTS AND DISCUSSION

3.1 Transducers

To evaluate the surface structure of prepared silicon-based electrodes, optical and electron microscopy was employed. The pictures from electron microscope showed the heterogeneity of the electrode in the case of SPG sensors and the smoothness and integrity in the case of BSC and TES structures (Figure 2). For the classical gold disc electrode, only optical pictures were taken (data not shown), showing its significant unevenness, clearly visible even at relatively low (10x) magnification. As a consequence, the electrochemical area, measured using chronoamperometry in $K_4Fe(CN)_6/K_3Fe(CN)_6$ solution, was quite similar to geometric area for silicon-based transducers. Table 1 shows the roughness factor for each type of electrode used in the present study. It is evident that BSC and TES transducers have better defined electrode surface, as compared to commercially available GDE and SPG.

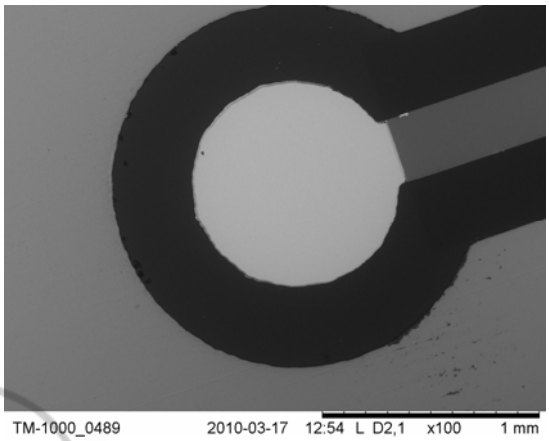

Figure 2: Electron microscope image of a working electrode of three-electrode structure (TES).

Table 1: Roughness factor for gold electrodes of transducers tested in this work.

Transducer	Roughness factor
GDE	3.45
SPG	2.61
BSC	1.75
TES	1.02

3.2 DNA Detection

To devise DNA biosensor constructed with fabricated BSC and TES transducers, the immobilization of ssDNA and subsequent hybridization with complementary strand was observed using impedance spectroscopy. The gold electrode was prepared according to procedure described in experimental section. Figure 3 shows impedance spectra of TES transducer with working electrode modified with ssDNA, as well as for the

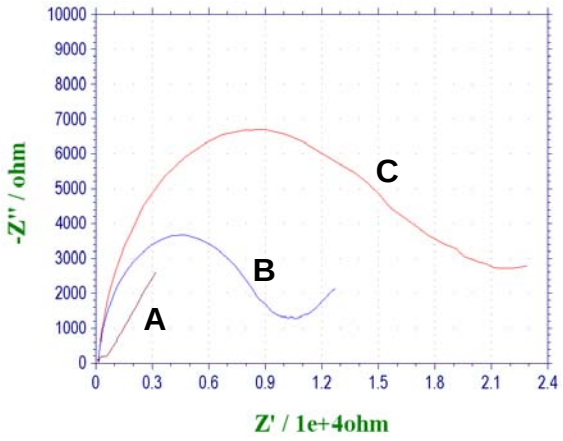

Figure 3: Impedance spectra of TES transducer with: A – bare gold electrode; B – DNA receptor layer; C – after hybridization.

same sensors after the hybridization. Based on these results, detection of hybridization event is evident.

Similar experiment could not be carried out for BSC sensors, most probably due to high impedance through the doped silicon structure. Accordingly, hybridization was detected using square wave voltammetry (SWV), using methylene blue as a redox marker. Again, obtained data confirm the immobilization of ssDNA on the gold surface, as indicated by redox potential shift ($\Delta E = 0.013V$). Further shift in redox potential ($\Delta E = 0.028V$) confirms that hybridization of receptor DNA layer with sample ssDNA takes place.

3.3 UO_2^{2+} Detection

It was reported recently that uranyl cation can cause DNA damage, especially in the presence of ascorbic acid (AA) (Yazzie et al., 2003). Many people can be potentially exposed to UO_2^{2+} through uranium mining, processing, the resulting mine tailings, and the use of depleted uranium in the military. Thus, determination of uranyl cation is very important from the clinical point of view. Based on the reported cleavage effect of UO_2^{2+}/AA on DNA, efforts were undertaken to devise uranyl sensor taking advantage of the degradation of DNA self-assembled monolayer, deposited on the BSC transducers. Surprisingly, UO_2^{2+} had no effect on the monolayer, even in the presence of ascorbic acid, as observed using impedance spectroscopy and SWV. Currently, work is in progress in our laboratory to elucidate this unsuspected behavior.

4 CONCLUSIONS

Silicon-based transducers with vacuum deposited gold were found to be useful for the construction of DNA sensors, mainly due to perfectly smooth surface of gold working electrode. Produced sensors, modified with oligonucleotide self-assembled monolayer, were shown to detect chosen DNA sequence. Efforts to determine UO_2^{2+} cation using the same sensors were unsuccessful.

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REFERENCES

Luong, J. H. T., Male, K. B., Glennon, J. D., 2008. Biosensor technology: Technology push versus market pull. In *Biotechnol. Adv.*

Andreescu, S., Sadik O. A., 2004. Trends and challenges in biochemical sensors for clinical and environmental monitoring. In *Pure Appl. Chem.*

Wang, J., 2000. *Analytical electrochemistry*, Wiley-VCH. New York, 2nd edition.

Hianik, T., Wang, J., 2009. Electrochemical Aptasensors - Recent Achievements and Perspectives. In *Electroanalysis*.

Erdem, A., Ozsoz, M., 2002. Electrochemical DNA Biosensors Based on DNA-Drug Interactions. In *Electroanalysis*.

Ziolkowski, R., Gorski, L., Zaborowski, M., Malinowska, E., 2010. Application of mass fabricated silicon-based gold transducers for amperometric biosensors. In *Bioelectrochemistry.*

Kelley, S.O., Barton, J. K., Jackson, N. M., Hill, M. G., 1997. Electrochemistry of Methylene Blue Bound to a DNA-Modified Electrode. In *Bioconjugate Chem.*

Yazzie, M., Gamble, S. L., Civitello, E. R., Stearns D. M., 2003. Uranyl Acetate Causes DNA Single Strand Breaks In Vitro in the Presence of Ascorbate (Vitamin C). In *Chem. Res. Toxicol.*