NanoBioPore: A novel nano-porous electrode system to enhance Biosensor sensitivity

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ABSTRACT

Electrochemical sensors comprising nanoporous metal-insulator-metal systems were fabricated and analyzed with respect to micro- and nanostructure, electrochemical redox-cycling and possible applications in medical diagnostics. An electrode spacing as small as 100 nm was realized and a sensitivity enhancement by a factor of up to 50 was demonstrated. Biological receptor molecules were immobilized on electrode surfaces via thiol residues or linked to pore walls employing epoxy-silane coupling. First results obtained using serum samples show low non-specific binding and high sensitivity.

Keywords: nanoporous electrodes, biosensor, diagnostics, redox cycling, electrochemistry

1 INTRODUCTION

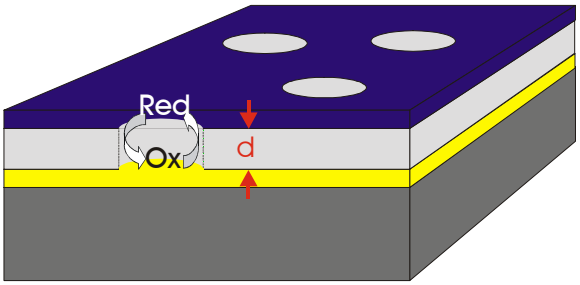

Figure 1: Schematic depiction of sensor structure and signal amplification by redox cycling. Dark grey: silicon substrate, yellow: bottom electrode (nanopore bottoms), light grey: insulator with d indicating insulator thickness and effective electrode spacing, marine: top electrode

The primary objective of this research is to create electrode systems for use as electrochemical transducers with enhanced sensitivity in order to enable detection of low concentration diagnostic targets not accessible to date. Redox cycling on interdigitated electrode arrays has been employed earlier to enhance sensitivity of electrochemical assays (*1*), (*2*), (*3*). However, a high amplification ratio may only be achieved by employing an electrode spacing below 1 µm. So far, this required costly UV- or e-beam lithography equipment, rendering such biosensors too expensive to allow for widespread application in the cost sensitive diagnostics market. In contrast, NanoBioPore sensors (Fig.1) consist of micro- or nano-porous metal-insulator-metal layer systems and exhibit an effective electrode spacing of only 100 nm between top and bottom electrode.

2 MATERIALS & METHODS

2.1 Chip fabrication

Sensors were fabricated on silicon wafer substrates (Fig.2). The bottom electrode was deposited by vapor deposition of titanium (10 nm), gold (200-300 nm), and titanium (10nm) through a lift mask. Subsequently, an insulating layer of SiNx of 100 – 200 nm was fabricated by plasma enhanced chemical vapor deposition.

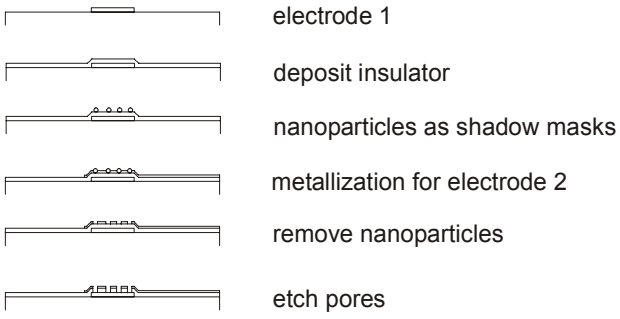

Figure 2: Fabrication of sensors using thin film technology and nanoparticle lithography. Particles serve as shadow masks to create a porous second electrode, which is also used as an etching mask to generate pores in the insulating layer.

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An ultra thin adhesion layer was applied by immersion in a 0.1% aqueous solution of polyethyleneimine rendering the surface of the insulator positively charged. A suspension of latex particles was applied to this surface for 10 min and rinsed with ultra pure water resulting in a monolayer of particles. Particle-particle spacing due to electrostatic repulsion is preserved while this layer is still wet. Upon drying, however, particles tend to form 2D-aggregates. In order to still ensure a laterally connective metallization in the following deposition step, two methods have been devised. By etching of the particle layer in an oxygen plasma particle size is reduced thus generating spaces between particles. Alternatively, particle layers were freeze dried, conserving a pattern with equally spaced particles (Figure 3).

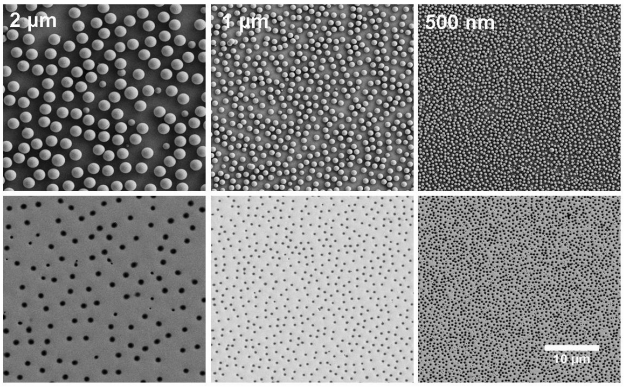

Figure 3: Particle lithography: micrographs show patterns of particles created by freeze drying sensors after immersion in a particle suspension and porous metallization layers obtained thereof. Numbers indicate diameter of particles employed.

Subsequently, electrode 2 was formed by plasma deposition or evaporation of a layer of titanium (10 nm) followed by gold (100 nm). Particles are removed by sonication in water, dried. Pores are formed by plasma etching in CF_4.

2.2 Biological assay development

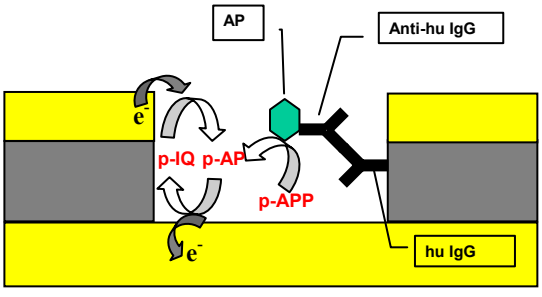

Figure 4: Receptor-ligand binding assay with an AP-modified detection antibody (anti-hu-IgG) recognizing a human IgG which was immobilized on the pore walls via epoxy-silane chemistry.

The NanoBioPore sensor system will support any biochemical assay (e.g. protein, DNA) resulting in the formation of a redox active compound that may undergo redox-cycling. Alkaline phosphatase (AP) coupled to a detection antibody will convert redox inactive para-aminophenylphosphate (p-APP) into redox active para-aminophenol (p-AP). Coupling of receptors to the sensor was achieved using either thiolated compounds binding to gold electrodes or employing an epoxy-silane which directs the immobilization to the pore walls (Figure 4).

3 RESULTS

3.1 Morphology and electrical properties

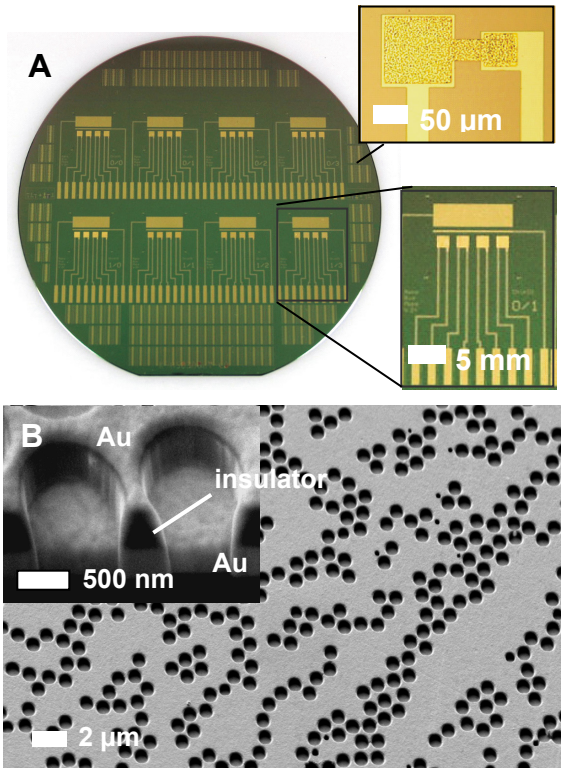

Figure 5: A) wafer, individual sensor chip comprising 4 sensor elements and enlarged view of sensor spot with porous area. B) SEM micrograph of a NanoBioPore electrode with 0.8 µm pores. The cross-section (insert) shows the metal / insulator in effect creating systems with very low electrode spacing

Pore morphology in sensor chips (Fig. 5A) was investigated by scanning electron microscopy. Cross-sections of layer systems were prepared by focused ion beam etching and also analyzed by SEM (Fig. 5B). These results were used to improve critical process steps such as

metallization, deposition of insulating layers and etching of pores in particular. Considering the combined perimeters of all pores of a sensor of several centimeters and the minute distance between top and bottom electrodes, the importance of well controlled surface properties of the pore walls is obvious. In table 1, electrical properties of sensors are compiled.

insulation layer thickness [nm]	capacitance [pF/mm^2]	leakage current [pA/mm^2]	number of sensors tested
100	670 +/- 118	63 +/- 17	218
200	384 +/- 32	62 +/- 18	48

Table 1: Electrical properties of sensors consisting of a gold / SiNx / gold layer system.

3.2 Electrochemical redox cycling

The degree of amplification by redox cycling achievable with NanoBioPore sensors was tested in the following way (Figure 6): the bottom electrode of a sensor was kept at reducing potential (-400 mV, constant potential amperometry) while the potential at the top electrode was increased from –400 mV towards more and more oxidizing potentials in steps of 50 mV. Arrows indicate potentials in mV applied at the top electrode. Electrolyte was 5 mM $[Ru(NH_3)_6]Cl_3$ in 100 mM KCl. A more than 50-fold increase of reducing current (red arrows at 15 s and 120 s) is measured when bottom and top electrode are biased to reducing (-400 mV) and oxidizing (+100 mV) potentials, respectively, thus demonstrating highly efficient redox cycling.

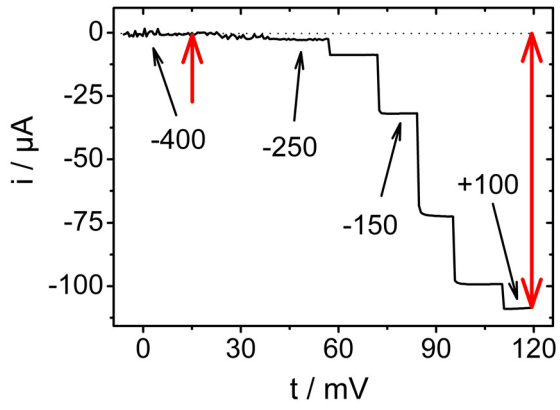

Figure 6: amplification by redox cycling in NanoBioPore sensors measured by current recorded on the bottom electrode upon increasing the voltage of the top electrode from -400 mV to +100 mV, thus essentially turning on redox cycling.

This is a significant improvement when compared to redox cycling factors reported from measurements using conventional IDE systems ranging between four- (*4*) and ten-fold (*5*). Further improvements with NanoBioPore systems are expected with optimized pore dimensions (*6*).

3.3 Receptor-ligand binding assays using NanoBioPore Sensors

In a first experiment, intended to explore NanoBioPore sensors for use with receptor-ligand binding assays, a series of dilutions of biotinylated alkaline phosphatase ranging from 84 fM to 8,4 pM was applied to a streptavidin coated microtiter plate. Para-aminophenyl-phosphate was added to the plate at a concentration of 0,5 mM. After 10 min, the solution was retrieved from the well and the concentration of p-AP generated by enzyme activity was measured using the NanoBioPore sensor (electrode spacing 100 nm, pore diameter: 850 nm). Figure 7 shows that the current signal is enhanced by a factor of 20 by redox cycling (◊) over the current measured without amplification (Top electrode at open circuit potential (□). The limit of detection obtained for biotinylated AP was 1.5 pM and the limit of quantification was 4.9 pM.

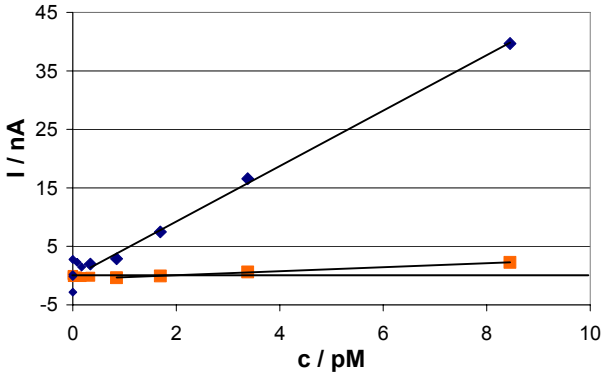

Figure 7: Biotinylated-AP was immobilized on streptavidin modified microtiter plates at the concentrations indicated in the graph. After 10 min of reaction time, the solutions containing p-aminophenol generated by the enzyme were retrieved from wells and measured. Clearly, redox cycling (◊) enhances signal intensity significantly over currents measured without amplification (□).

A human-IgG-antibody was selectively immobilized on the pore walls via epoxy silane. The binding of an anti-human-IgG antibody conjugated with alkaline phosphatase was detected by chronoamperometry (Figure 8). The assay was shown to be functional by the detection of p-aminophenol generated by the enzyme. The NanoBioPore sensor could detect immobilized primary antibody at dilution ratios of the secondary antibody of up 1:20,000 (whilst available reference serum assays employ typical

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ratios of 1:40 to 1:100). Even though somewhat preliminary, these results confirm the compatibility of this sensor with serum samples.

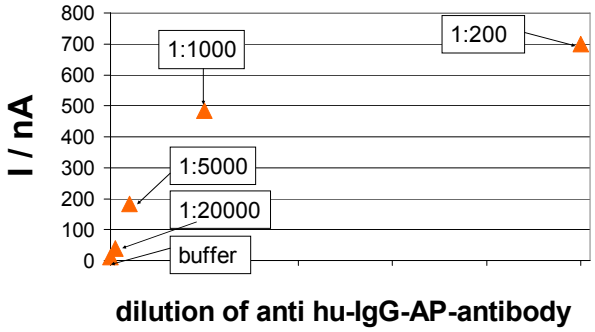

Figure 8: Direct binding assay comprising primary hu-IgG-antibody (c = 0,1mg/ml), immobilized on the pore walls of NanoBioPOre chips, and secondary anti-hu-IgG-AP-antibody as a detector. The anti-hu-IgG-AP-antibody was applied at different dilutions resulting in a dilution-dependent redox cycling-signal at the bottom electrode

4 CONCLUSIONS

The effectiveness of nanoporous electrode / insulator / electrode systems (NanoBioPore) for sensitive electrochemical detection employing redox cycling in micro- or nanopores has been demonstrated. Amplification factors superior to state of the art IDE electrodes have been measured. Since fabrication of these devices does not require any high definition lithography, cost sensitive diagnostic applications addressing low concentration biological targets become accessible.

Future research will be directed towards further optimization of pore geometry and density with respect to redox cycling efficiency and the establishment and validation of a panel of diagnostic assays.

5 ACKNOWLEDGEMENT

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