SYNTHESIS OF GOLD NANOELECTRODE ENSEMBLES FOR BIOSENSING APPLICATIONS

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ABSTRACT

Metal nanowires were synthesized according to an electroless deposition method in polycarbonate template, obtaining nano electrodes ensembles with specific features and well defined diameter size. Nano electrode ensembles were coupled with disposable screen printed substrate in order to increase the sensitivity of the commonly used carbon graphite working electrode for electrochemical sensing. Furthermore, working surface modification provided the basis for original protein immobilization procedures that otherwise would not be allowed.

Keywords: nanoelectrodes ensemble, screen printing, electrochemical biosensors, glucose monitoring, immobilization

Abbreviations. APTES: amino propyl triethoxy silane; CYS: cysteamine; FIA: flow injection analysis; GA: glutaraldehyde; GOx: glucose oxidase; l.o.d.: limit of detection; NNE: nanoelectrode ensemble; PAN: polyaniline; PB: phosphate buffer 0.1M (pH=6.8); PC: polycarbonate; RE: printed Ag/AgCl pseudo-reference electrode; SAM: self-assembled monolayer; SPE: screen printed electrode; SPS: screen printed substrate; WE: working electrode.

1. INTRODUCTION

Nanostructured materials have proven as one of the most powerful tools in new technologies and research, due to their peculiar properties at nanometer size scale. Many studies have shown that optical, mechanical, photo-catalytic and transport properties drastically changes, depending on quantum size effect, as the mean diameter of the particles is in the exciton size regime [1-5]. Both metallic and semiconductor nanosized materials have found large applications in biochemistry, bioanalytical techniques as well as luminescence, catalysis, optoelectronics and photochemistry [6]. The unique chemical/physical properties of metal, oxide and semiconductor nanoparticles make them extremely suitable also for designing new and improved sensing devices, especially electrochemical sensors and biosensors. The important functions of nanostructured particles include the immobilization of biomolecules, the catalysis of electrochemical reactions, the enhancement of electron transfer mechanism between electrode surfaces and proteins, labeling of biomolecules and even acting as reactant. The topic task for such a biomolecules immobilization is the capability to assemble the biochemical recognizing system on such sensors, controlling at the same time the surface activation (i.e. using SAM) and the possible substrate modification [7-13].

NNE were synthesized in PC template according to an electroless deposition method [14,17]. Au Nanowires were obtained with the expected diameter size and coupled with carbon SPE. The association of Au NEE with graphite substrates is aimed to couple the high electroanalytical sensitivities, deriving from the nanosized properties, with the feasibility and versatility of screen printing technology in easy to be used sensors. NEE based biosensors were compared to unmodified carbon and Au SPEs.

2. EXPERIMENTAL

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2.1. Preparation of Screen Printed Substrates

Conducting and insulators inks were printed on 0.3–0.5 mm thick polyvinyl-chloride (PVC) substrate using a HT10 Fleischle\textsuperscript{TM} screen printer (Brackenheim, Germany). Silver and carbon-graphite pastes for the conducting paths and WE, Ag/AgCl for RE and insulator pastes were all from GWENT Electronics Materials Inc\textsuperscript{TM}. SPEs based on conductive pastes from GWENT were shown to be useful for batch or flow-through measurements where high quality of electrochemical response is required.

2.2 Synthesis of Au nanowires

The metal, semiconductor, oxide and polymer nanostructures growth into template systems represents a simple and rapid synthetic route, especially using nanoporous PC or alumina membranes. Electroless deposition of Au was performed into track etched PC membrane (Unipore\textsuperscript{TM}, nominal pore size 30 nm, thickness 10 µm) according to Menon and coworkers\cite{14}. Such membranes are preferred for NEE fabrication over alumina membranes because of their smaller pore densities and their lower fragility. The synthesis took place, immersing the membrane 40 min for the surface activation by Sn (tin chloride solution was from Sigma Aldrich\textsuperscript{TM}), 15 min for Ag nanoparticles deposition (silver nitrate from Sigma Aldrich\textsuperscript{TM}) and using AuCl\textsubscript{4}\textsuperscript{-} solution (Sigma Aldrich\textsuperscript{TM}) for Au deposition by means of galvanic displacement\cite{14}. Scanning electron microscope (SEM) observations, as in figure 1, show the successful formation of 30 nm diameter Au nanowires with a good size distribution (image obtained after removing the front side of macroscopic Au layer grown over the front side of the template membrane). From the figure, nanowires density can be also calculated of around 6 pores µm\textsuperscript{-2}, in accordance with the declared pore density (6 \times 10^8 pores cm\textsuperscript{-2}).

![Figure 1: Scanning electron microscopy (SEM) image of Au nanowires deposited into nanoporous PC membranes (nominal pore size: 30 nm)](image)

2.3 Nanoelectrode ensembles on screen printed substrate

Au nanowires modified PC membranes can be either used as platform for immobilization of biomolecules or as free standing NEEs, i.e. a large assembly of very small ultramicroelectrodes confined in a rather small space. In each case, NEEs can exhibit three distinct voltammetric response regimes, depending on the scan rate and reciprocal distance between the nanoelectrode elements\cite{14-17}: total overlap regime, pure radial regime and linear regime. It was demonstrated that for electroanalytical applications the total overlap regime is the most advantageous one because of the higher faradaic-to-capacitive current ratios, resulting in a signal to noise ratio 10\textsuperscript{2} higher than conventional Au macro electrode with comparable geometrical area\cite{14,16}. In order to assemble our nanoelectrodes based membrane with SPS (obtaining NEE/SPS), Au NEE membrane was one side peeled and soaked only on the rear side into a wet graphite ink pad. Either a vacuum pen or a vacuum-controlled silicone rubber tube were conveniently used for placing the inked membrane on the graphite WE. Afterwards, the device was completed by printing insulator for a resulting working area of 2.54 mm\textsuperscript{2} and RE layers as in common screen printing procedure.
2.4 Materials for NEE/SPS characterization

A home made flow cell was used for the characterization of NEE/SPS and the amperometric detection of glucose with a GOx based biosensor. The microcell was tailored to tightly lodge the device in between two Perspex blocks, leaving two holes in the upper side equipped for connection to the sample loop (115 µL) via Teflon tubing. The peristaltic pump (Minipuls-3 from Gilson™) was used to propel solution along a FIA system. PB was used as a carrier in all the following experiments. Amperometric experiments performed under batch and FIA conditions were conducted with the Autolab™ potentiostat PGSTAT10 and homemade Domotek™ instrumentation.

3. RESULTS

3.1- FIA response in nude NEE/SPS and NEE/SPS glucose sensor

GOx immobilization was used as a model system to test feasibility and analytical performances of the sensing probe. The unmodified NEE/SPS was firstly tested with specific substrate of GOx. No response was detected, in this way excluding the occurrence of non-specific oxidation of glucose at Au surface. Two main GOx immobilization techniques were experimented:

- GOx covalently immobilized via cysteamine self assembled monolayers and glutaraldehyde (GA) on golden probe (GOx/SAM/NEE/SPS). CYS was assembled on Au nanodisks either electrochemically or chemically [18]. The probe was immersed for 1 hour in a 12.5 % v/v solution of GA and accurately washed with buffer; then GOx solution (6 mg mL⁻¹) was dropped on NEE/SPS and left to be covalently attached to the carbonyl group of the coupling agent. The non-covalently bound enzyme as well as the excess of GA was easily removed by a three-fold washing process.

- GOx covalently immobilized on the membrane via APTES and PAN grown on nanowires part (PAN/GOx/NEE/SPS).

FIA conditions were optimized for the highest current signal: the optimal flow rate was reached at 0.4 mL min⁻¹, using a sample loop of 115 µL (data not shown). Signal to background ratio as well as the response of current were measured as a function of the applied potential, due to the oxidation of the produced H₂O₂, after multiple injections of 10⁻³ M glucose. The highest response were obtained in each case within the range +550÷650 mV. Potential of +650 mV was applied in further experiments.

3.2- Performance of glucose sensors

- GOx/SAM/NEE/SPS. The response of the presented biosensor to glucose concentration was investigated between 7.5 10⁻⁶ M and 3.1 10⁻² M (R²=99.3%), within the analytical range for blood monitoring. At higher glucose concentration saturation of enzymatic active sites took place and response was no more linear. The sensitivity for GOx/SAM/NEE/SPS was 34.6 nA mM⁻¹ glucose, l.o.d. of 1.5 10⁻⁴ M was calculated by Zund Meier method [19]. The linear response can be estimated by Michaelis Menten analysis of the calibration plot: an apparent constant of 14.9 mM (lower than that of the native enzyme, 33 mM [20]) was obtained after extrapolation of the Lineaweaver-Burk relation. High operational stability was also achieved within 48 hours of continuous glucose injection and shelf lifetime, expressed as the period corresponding to the 90% of the initial response, was 20 days.

- PAN/GOx/NEE/SPS. The biosensor made of PAN wires on NEE with GOx covalently immobilized was tested under FIA conditions above described. The response of such a biosensor is linear (R²=99.8%) in the concentration range of glucose from 10⁻⁵ M to 4.0 10⁻² M. The sensitivity was 18.2 nA mM⁻¹ glucose and l.o.d. was 1.5 10⁻⁵ M. Apparent constant of 8.9 mM was obtained. Shelf lifetime at 90% of response was 15 days. The achieved long term stability for PAN/GOx/NEE/SPS biosensor is a confirmation of the role of silanizing agent to react with not golden part of the template and consequently, to allow covalent bond between enzyme and surface. Figure 2 shows the comparison of the calibration curves of GOx/SAM/NEE/SPS (triangles) and PAN/GOx/NEE/SPS (dots); the inset shows a normalized plots of chronoamperometric FIA response for both sensors at the concentration level of 0.5 mM glucose. The lower response and return times relatively to PAN/GOx/NEE/SPS biosensor (dotted line) could be due to the direct electron transfer between the PAN wires and the electrode surface.
Figure 2: calibration curve for glucose sensor based on GOx/SAM/NEE/SPS (triangles) and GOx covalently immobilized on the membrane (dots); INSET: FIA responses for the proposed biosensors. Solid line, GOx/SAM/NEE/SPS. Dotted line, PAN/GOx/NEE/SPS.

Analytical performances were compared with those available from literature in similar enzyme systems based on Au NEEs [21]: although in a less extent with, detection limits for glucose are still not comparable with the NEE features. This partial loss of the nanoparticles properties may be ascribed to the number of layers grown on the surface during the immobilization procedure.

4. CONCLUSIONS

Nanoelectrodes ensembles (NEEs) were prepared by using the electroless deposition of Au nanowires into nanoporous template membrane. Fabrication of screen printed substrate (SPS) couple to nanosized component resulted in an original system for biosensor applications: NEE/SPS. NEEs/SPS were tested under flow in a glucose oxidase based sensor. High stability and linearity of the electrochemical response of the overall system were successfully achieved. According to this novel approach, the advantage of nanostructured material properties (higher sensitivity than conventional electrode, capability to control the property depending on the nanoparticles size) was enhanced with features of thick film technology, such as disposability, flexibility, durability of the product.

REFERENCES