GOLD NANOCOMPOSITE FOR FOOD SAFETY

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Introduction

The oriented and improved immobilization of DNA to solid substrates has gained importance over the past decade, due to its use as a genomic detection tool in DNA arrays[1], DNA biosensor[2] and self-assembled molecular electronic circuits[3]. Owing to the strong affinity of thiols to metal surfaces, sulfur chemistry is widely employed when attaching DNA, especially to continuous gold films[4] or, instead, to gold nanoparticles-modified electrodes, to increase the electroactive surface.[5] Although van der Waals attraction drives the assembly and ordering in typical SAMs, DNA immobilization is subject to strong electrostatic repulsion.[6] As a result, in hybridization experiments, although a spacer arm being used, tightly packed and negatively charged SAM are obtained, which impedes hybridization with the complementary DNA probe due to both steric as well as electrostatic effects.[7] As such, a stringent control of the surface coverage of DNA is an important factor in maximizing hybridization efficiency which can be performed by using auxiliary reagents such as lateral spacer thiols and mixed monolayers to obtain bioactive gaps. [8]

In order to avoid the stringent control of surface coverage parameters during immobilization of thiolated biomolecules on continuous gold films, the use of gold nanoparticles in a graphite-epoxy composite (nano-AuGEC) is presented in this paper. Islands of chemisorbing material (gold nanoparticles) surrounded by rigid, non-chemisorbing, conducting, graphite epoxy composite are thus achieved. With this arrangement in the electrochemical transducer, the resulting less-packed surface provides improved hybridization features with a complementary probe minimizing steric and electrostatic repulsion. Rigid conducting gold nanocomposite represents a simple method for the oriented immobilization of biomolecules. The microscopic and electrochemical characterization of this material is presented in this paper, as well as its utility in electrochemical genosensing for food safety.

Experimental section

Instrumentation

The Leica MZ FLIII fluorescence stereomicroscope (Leica, Heidelberg, Germany) and the Jeol JSM-6300 scanning electron microscope (Jeol LTD, Tokio, Japan) coupled with an EDX detector which allows a characteristic X-ray spectrum (Oxford instruments, Bucks, England) were used to study the distribution of the gold nanoparticles on the electrode surface.

Amperometric measurements were performed with a LC-4C amperometric controller (BAS Bioanalytical Systems Inc., USA). Voltammetric characterization was carried out using an Autolab PGSTAT Eco-chemie (The Netherlands).

Construction of the nanoAu-GEC electrodes

For GEC electrodes, graphite powder and epoxy resin in a 1:4 (w/w) ratio was thoroughly hand mixed to ensure the uniform dispersion of the graphite powder throughout the polymer. The designation of the electrodes is based on the ratio of gold nanoparticles towards graphite particles. In all cases, the resulting paste was placed in a PVC cylindrical sleeve body (6 mm id), which has an electrical contact to a depth of 3 mm.

After filling the electrode body gap completely with the soft paste, the electrode was tightly packed. The composite material was cured at 80 °C during one week.

Results and discussion

Microscopic characterization of nanoAu-GEC electrodes

The location and spatial pattern of the gold nanoparticles on the surface of the sensor was observed with scanning electron microscopy with EDX detector. High resolution SEM micrograph shows clearly isolated gold nanoparticles of about 100 nm within the composite, demonstrated with the EDX detector providing the characteristic gold X-ray spectrum. Moreover, the availability of gold nanoparticles in the composite for the

immobilization of thiolated oligos was also study with fluorescence stereomicroscopy. In this case, 200 pmol of double-tagged oligo with both a thiolated 5' end and the fluorescein 3' end was immobilized on the electrodes with different composition. An increasing amount of fluorescence was obtained with higher amount of gold nanoparticles in the composite. The fluorescence shows a discontinuous pattern except in the case of nanoAu(100%)-EC, in which a continuous fluorescence pattern is clearly observed. Moreover, it should be point out that the fluorescence can be related with the isolated gold nanoparticles pattern because it is not located in the aggregates zones, when comparing with the same photos taken with the stereomicroscope without the fluorescence filter.

Electrochemical characterization of nanoAu-GEC electrodes

NanoAu(7,5%)-GEC electrode showed very similar electrochemical behaviour compared with non-modified GEC (nanoAu(0%)-GEC) by cyclic voltammetry using HQ (the HRP mediator) as a electrochemical reporter. The relationship Ipc/Ipa is equal to unity. When increasing the amount of gold nanoparticles, the electrochemical signal showed to be worsened, with displacement of values of peak potential and decreasing of signal current intensity, perhaps due to the increasing amount of gold aggregates The cyclic voltammograms of nanoAu(7,5%)-GEC is overlapped with the GEC electrode, except by the oxidation of gold at 1,1 V, with is maintained almost equal after cycling 10 times.

Although higher electrochemical signals were obtained with the acid and electrochemical treatment of the surface, the polishing treatment was found to be the most reproducible. Eight different nanoAu(7,5)-GEC electrode were polished and studied by cyclic voltammetry, showing SD(%)= 6,7, for anodic current in 1.81 mmol L^{-1} of hydroquinone in phosphate buffer 0.1 mol L^{-1}, KCl 0.1 mol L^{-1}, pH 7.0. (V= 100 mV s^{-1}).

Conclusions

The nanoAu-GEC material shows interesting properties for electrochemical genosensing in hybridization experiments, and very promising features for electrochemical biosensing of a wide range of biomolecules, such as dsDNA, PCR products, affinity proteins, antibodies or enzymes. Instead of SAMs on continuous layer of gold, isolated gold nanoparticles are able to produce 'bioactive chemisorbing islands' for the immobilization of thiolated biomolecules, avoiding stringent conditions for surface coverage as well as the use of auxiliary reagents such as lateral spacer thiols. Less compact layers are thus achieved favouring the biological reaction on biosensing devices. The spatial resolution of gold nanoparticles was demonstrated to be easily controlled by merely varying its percentage in the composition of the composite. Furthermore, the chemisorbing ability of gold nanoparticles in the nano-AuGEC was demonstrated for the detection of as low as 50 fmoles of ssDNA in hybridization studies. The rapid electrochemical verification of the amplicon coming from the pathogenic genome of *Salmonella* performed by PCR with a set of two labelled primers demonstrated to be an easy way for the thiolation of the PCR product for food safety. The thiolated end allowed the immobilization of the amplicon on the nano-AuGEC electrode in an easy way to be electrochemically detected at fmole concentration range.

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