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Metallic nanowires – unconventional tools for fluorescence-based biomedical applications

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Metallic multilayered nanowires based on gold and platinum were synthesized by pulsed electrodeposition. The free nanowires were impurity-free, with some fine branch-like shapes and non-uniform final lengths. The in-membrane sizes of the nanowires were up to 25 μm in length, and about 200 nm in diameter. The nanowires were the subject of a pathological DNA immobilization, via specific PCR (Polimerase Chain Reaction) amplification, in order to allow the fluorescence-based detection of the specific DNA sequence.

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1. Introduction

Metallic nanowires, including noble metals (e.g. gold or platinum), have attracted significant interest because of their optical properties, biocompatibility and resistivity properties [1-4].

Also, detection and quantification of biological and chemical species, representing a central goal for many areas of biomedicine, take a progressive advantage from nanomaterial technology development. The applications of nanomaterials, such as nanowires, nanoparticles [5], carbon nanotubes, in biomedicine can solve a series of specific problems related to diagnosis or therapy [6].

In the last ten years, nanowires were shown to have a high potential to be used in biomedical applications, especially for diagnosis and magnetic separation purposes. Thus, the nanowires were tested in biosensors [7], bioassay methods for pathogen detection [8] or magnetic manipulation of cells [9].

The capability to use nanowires as carriers for various antibodies and fluorescent labels, due to progresses in surface chemistry, has enabled their successful use also in drug delivery and biosensing [10]. Gold nanowires were used for enzyme biosensors because gold-nanowires-based electrodes provide very large surface area per unit volume, allowing the immobilization of higher amounts of specific biomolecules [11]. In addition, nanowires with sub-micron diameters have also been utilized to study cell biology [12]. Furthermore, nanowires were used as supports and carriers in immunochromatographic assay of human antigens [13].

Moreover, a direct, rapid, and label-free electrochemical immunoassay method for testosterone antibody has been described based on a sol–gel film doped with gold nanowires [14]. When compared with conventional ELISAs, this immunoassay method was showed to be simpler and rapid without multiple labelling and separation steps that are specific for ELISA method.

From the medical analysis standpoint, the most important biomolecules used for diagnosis are antibodies and DNA. The discovery of specific target DNA sequences of medical interest in the incipient phase of a disease such as tumor or viral pathology is correlated with an accurate assessment of patient's prognosis and with an appropriate way to monitor therapy. Usually, these specific DNA sequences are detected and quantified using molecular techniques (PCR, RFLP, RT-PCR) along with electrophoretic migration in agarose gel. Regarding this issue, an alternative technique to the conventional ones could be the use of a bioassay method based on metallic nanowires that specifically detect and qualitatively identify the amplified target DNA sequences obtained by using specific modified primers.

Following the general tendency of nanowires' applications in biomedical domain, this study presents our results concerning a convenient method of synthesis of gold-platinum nanowires by electrochemical deposition and use of nanowires to detect and qualitatively identify a DNA sequence obtained from patients with acute myeloblastic leukemia.

2. Experimental

The nanowires were prepared by electrochemical deposition of gold and platinum ions respectively [15], into the pores of a membrane template, followed by nanowire release. Concretely, the nanowires were growth inside the anodic aluminium oxide (AAO) template provided by Whatman. These templates have a specific pore size of 200 nm and a thickness of 50 μm (Fig. 1).

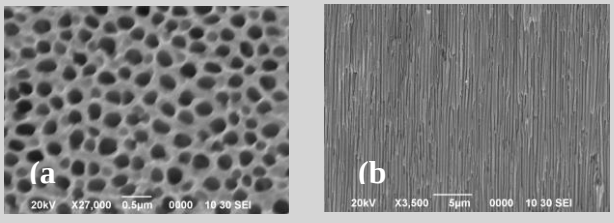

Fig. 1. SEM micrograph of Whatman nanoporous Al_2O_3 membrane (a) top view; (b) section view.

For electrochemical deposition of metals, we used a three-electrode cell: SCE was used as reference electrode whereas Pt foil functioned as counter electrode. Prior to electrodeposition, an adhesion layer of Au film, a few hundred nanometres in thickness, was sputtered by thermal evaporation onto the backside of the AAO template in order to completely cover the nanopores, and to serve as the working electrode during electrochemical deposition. All our experiments were performed at room temperature by pulsed electrodeposition [6]. The preparation of multilayer Au/Pt nanowires was carried out by successive deposition of the gold and platinum ions that came from two different electrolytic solutions. Platinum nanowires were grown in aqueous solution of H_2PtCl_6 5 mM/L and HCl 0.1 M by applying a d.c. current of -0.2 V for 3 s. In the case of gold nanowires deposition, an aqueous solution of $HAuCl_4$ 5 mM/L and H_3BO_3 0.5 M was used. The electrodeposion was performed by applying a d.c. current of -1.3 V for 5 s.

It is worthy to add here that, at this stage of synthesis, the dimensional aspect of the nanowires can easily be controlled by (i) the membrane pore diameter, which dictates the wire's width, and (ii) altering the current and deposition time, which set the particle length.

The nanowires were removed from the template according to a generally used procedure [16]. Thus, after dissolving the alumina membrane in 1M NaOH, the solution was heated for 10 min and then sonicated in an ultrasonic homogenizer (Hielscher UP50H, Germany). The hydroxide solution was renewed several times with a freshly prepared 1M NaOH one in order to make sure the AAO template was completely dissolved and the gold-platinum nanowires were released in solution. Thereafter, the nanowires were washed several times with distilled water in order to remove the last traces of NaOH and separated by using a centrifugal process (Sigma 3-18K, Sortirius ultracentrifuge). The final pH of stock solution was 6.5, confirming the NaOH was completely removed.

3. Results and discussion

After electrodeposition, the cross section of the AAO template, filled with noble metals, were characterized by scanning electron microscopy (SEM) by using a JEOL microscope. Fig. 2 shows a SEM micrograph of the cross section of the AAO filled with gold-platinum nanowires at two different scales.

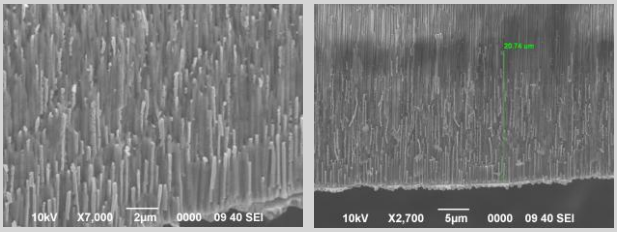

Fig. 2. SEM picture of the membrane cross section after successive gold and platinum electrochemical deposition.

The analysis of the images shows membrane is homogeneously filled with noble metals. It was additionally observed that the growth rate of nanowires was changing in function of the nature of the electrodeposited metals. Thus, for platinum deposition, the growth rate was 4 μm/h whereas for gold was 10 μm/h. After deposition, a piece of AAO membrane, filled with nanowires, was analyzed by EDS (Fig. 3).

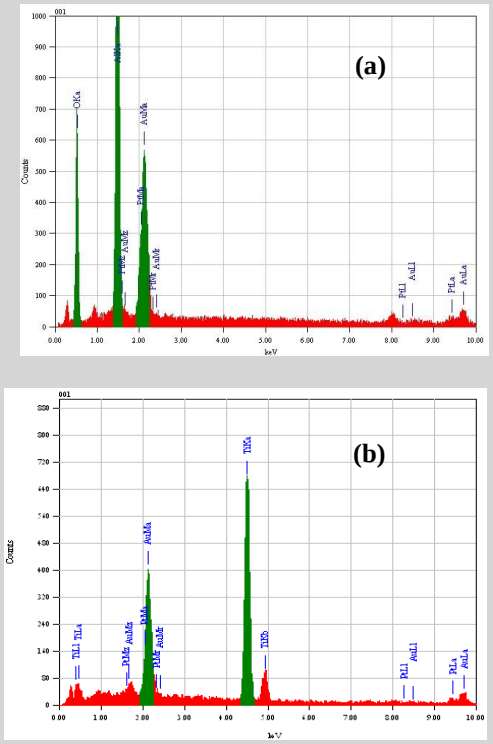

Fig. 3. EDS analysis of gold-platinum nanowires: (a) nanowires in membrane and (b) free nanowires.

The remains of the AAO template, after following the above presented procedure for nanowire delivery, were finally washed with a diluted solution of HCl in order to remove any potential trace of impurities. Subsequently, they were analyzed by EDS. The EDS spectra (Fig. 3) show the obtained nanowires do not contain any impurity. However, there are apparent impurities consisting in oxygen, aluminium, and titanium that are well represented in the EDS spectrum. The immediate explanation is related to the oxygen and aluminium species that form AOO template, whereas titanium comes from the sample holder, the free nanowires being settled directly on the titanium holder. Therefore, the nanowires are pure form the compositional standpoint.

The sizes and shapes of the free nanowires were analyzed with SEM at 10 kV (Fig. 4). As in the case of the pictures taken from the cross-sectioned filled membrane, one can observe also in the SEM images of free nanowires, the two different microstructure specific to platinum and respectively gold nanowires.

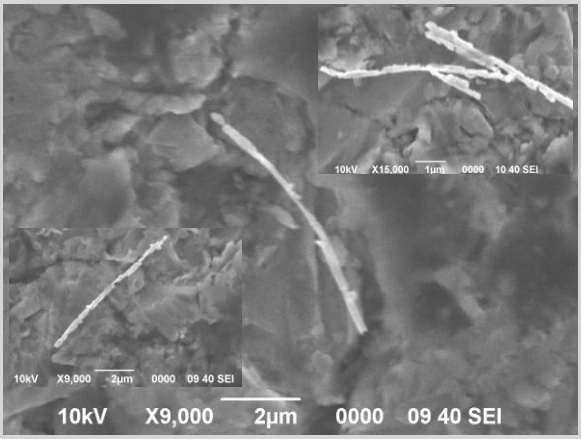

Fig. 4. SEM images of different naked gold-platinum nanowires.

As Fig. 4 shows, the free nanowires do not present perfect smooth surfaces, and some small, but well visible, branches emerge from the main body of the nanowires. It is also observed that even the original sizes in membrane were at least 7 micrometres, the sizes decreased after the wash and centrifugal processes caused their fracture and split them in small pieces. However, even split, the nanowires contain both platinum and gold layers.

Nanowire-based detection of specific-desease DNA from a patient suffering from acute mieloblastic leukemia

To test their ability to be used in a biodetection assay, the naked gold-platinum nanowires were further on utilized for identification of a DNA sequence, specific for FLT3 gene mutation, responsible for acute mieloblastic leukemia.

Given in a synthetic hierarchy, the main steps of the procedure were performed as followed:

1. Separation and purification of specific genomic DNA from the blood of patients with acute mieloblastic leukemia, in order to detect mutation of FLT3 gene;
2. Amplification of target DNA sequence through PCR (Polimerase Chain Reaction) amplification by using specific primers modified at their 3' ends with thiols, i.e. HS- chemical groups, simultaneously with other primers having their 5' ends modified with a fluorophore, i.e. cy5;
3. Immobilization of the obtained PCR products by using metallic nanowires and further detection through fluorescence-based analysis system;
4. For comparison, detection of the same PCR products was made by using a gel electrophoresis migration method.

In an additional point-to-point explanation, we should make clear some aspects related to the above synthetically presented steps of the procedure. First, the DNA separation from patients' blood followed a general and validated procedure [17-19]. Second, amplification of target DNA sequence was made in the presence of commercial primers, i.e. short single stranded DNA, specific to the "diseased" DNA. Third, due to the thiol groups, which quickly and specifically bind to gold surfaces, the amplified DNA was immobilized on gold-platinum nanowires. The nanowire-DNA structures were investigated through a fluorescence-based analysis system (Tissue Gnostics Faxs system) by measuring the fluorescence generated by the florophore-tagged amplicons (i.e. products resulted from PCR amplification process) immobilized on nanowires (Fig. 5).

Finally, in order to basically validate the method, a fluorescence-based gel electrophoresis migration method was used as a comparison tool for detection of the same PCR products (Fig. 5).

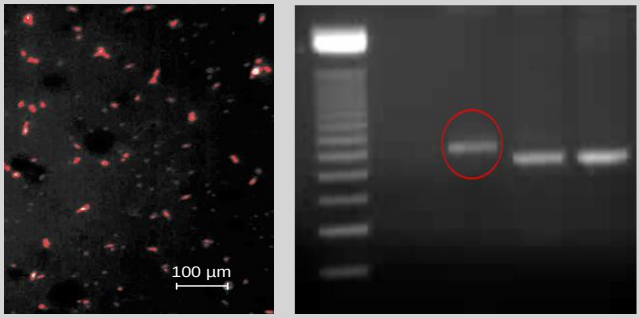

Fig. 5. (a) Nanowires detection through the fluorescence-based analysis system performed by measuring the fluorescence generated by the fluorophore-tagged PCR-amplified products immobilized on nanowires; (b) fluorescence-based gel electrophoresis migration of PCR-amplified products showing FLT3 gene mutation (inside the red circle).

From Fig. 5(a), red spots of DNA-nanowires complexes are well observed. As obviously can be seen, generally, the nanowires are not individually spread out between the two laminas of the microscope, but in small groups. This behaviour is due to the typical physical forces governing interactions in liquids. However, the image is convincing as regarding the successful immobilization and qualitative detection of DNA, specific to FLT3 gene involved in acute mieloblastic leukemia.

As an additional remark, the platinum layers in nanowires were intended to introduce an optical reflectance difference when different wave lengths of electromagnetic radiation are used, aiming to further simultaneously detection of different disease-specific DNA. However, at this stage of research, this additional possibility was not exploited due to some apparatus-related limitations.

However, in spite of this first successful work, based on a comparative study, and that emphasized the usefulness of the nanowires-based bioassay method for specific biomedical issues, further analysis and tests are needed in order to certify the efficiency, sensitivity and specificity of this method.

4. Conclusions

In a synthetic final analysis, we can objectively conclude that we managed to successfully prepare multilayered gold-platinum nanowires by pulsed electrodeposition. The obtained nanowires have been investigated by SEM and EDS. The results showed nanowires with diameters of

about 200 nm and lengths of several micrometers, being pure for the chemical analysis standpoint.

The nanowires were used to immobilize a disease-related DNA that was further detected by using a fluorescence-based analysis system. Also, the same disease-related DNA was detected through gel electrophoresis migration.

The comparative study showed the target amplified DNA was successfully detected by using metallic nanowires, the entire detection process being, in principle, simple.

The results also underlined the nanowires-based bioassay method could be used for specific biomedical assays in one condition: further analysis and tests in order to certify the efficiency, sensitivity and specificity of this method have to carry out. Accordingly, for further progress on this method, we will focus on this imposed condition, including other refinements of the presently described method.

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