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Synthesis and Characterization of Noble Metal Nanowires

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1. Introduction

In the ten last years the nanomaterials science and technology have represented one of the most attractive interdisciplinary science researches. The growing interest for the nanoscience domain resides in potential applications in physics, chemistry, biology and electronics. Nowadays, the research in the nanomaterials field takes advantage from important funding since they are the basis for the development of new technologies, devices and systems. Bibliographical data present many synthesis methods of simple and/or multilayered nanowires such as: photochemical synthesis (Kim et al., 2002), catalytical synthesis (Huang et al., 2002), vapour-liquid-solid growing (Björk et al., 2002), electrochemical deposition (Yu et al., 1997; Inguanta et al., 2009; Xu & Wang, 2008).

The preparation of nanowires by electrochemical deposition in nanosised pores is more frequently used because of the low cost and the better energetic efficiency of process. The electrodeposition is a preparation method which allows the controlled deposition from solution of metallic materials. Generally, such a solution contains dissolved salts of metals which are going to be deposited. Passing of a current through the electrochemical cell (formed by three electrodes: the reference electrode, the counter electrode and the working electrode) allows the ions migration from the electrochemical bath to working electrode and their deposition in metallic state. There are a large number of metals which can be deposited by using this method from aqueous solutions such as: Ni, Fe, Co, Ga, B, Cu, Cr, Zn, Ru, Rh, Pd, Ag, Au, Pt etc.

In the case of materials prepared by the electrochemical method, besides the condition that can be easily used for the process development, the quality of the synthesized material can be better controlled by fine-tuning the electrolyte composition and electrolysis parameters control such as: the applied potential, the current density, electrical charge, temperature and the type of the electrolysis (potentiostatic or galvanostatic). The electrochemical method allows the preparation of nanowires with a high length/diameter ratio in polymeric membrane or anodised aluminium oxide membrane (AAO). The localised growth of straight and parallel nanowires on plane surfaces is a specific geometric feature that can be used to obtain nanosized interconnections for electronic and magnetic devices.

In the case of electrochemical cell used to prepare metallic nanowires, the anode is a platinum foil and the reference electrode is the saturated calomel electrode (ESC), silver electrode/silver chlorine (Ag/AgCl) or graphite electrode.

The working electrode is the electrode whose surface is used as support for the ions reduction from the solution. In the case of the electrochemical deposition of simple or multilayered nanowires the nanoporous membrane, with pores which will be filled with metallic nanowires, is used as cathode in electrochemical cell. To be used for electrochemical deposition the membranes are prepared as follows:

- on one side of the nanoporous membrane a 500 nm gold thin film is deposited by thermal evaporation in vacuum;
- the gold layer is physically isolated from the electrolyte by using a special insulator layer.

In this configuration, the deposited metallic layer is not in direct contact with the electrolyte, but only through the pores of the membrane, the electrochemical deposition being achieved only by pores.

The preparation of metallic nanowires by using nanoporous membrane involves a better knowledge of physical and electrochemical processes of deposition.

Among the most used membranes for the preparation of nanowires are the polycarbonate membranes and the alumina membranes. The polycarbonate membranes are obtained by the "track-etch" method. This method uses the bombardation with heavy atoms of a nonporous material to create holes. This step is followed by chemical treatment to transform the holes in nanopores. The nanoporous membrane contains cylindrical pores of uniform diameters but which are randomly distributed on its surface. This type of membrane, commercially available (Nucleopore and Poretics companies), may contain pores with diameters between 10 nm and 800 nm with variable densities.

Alumina nanoporous plane membranes (AAO) are obtained by anodization of aluminium foils in acids electrolytes containing bivalent or trivalent anions such as: oxalic acid $(\text{COOH})_2$ (Li et al., 1999), sulphuric acid H_2SO_4 (Jessensky et al., 1998), or phosphoric acid H_3PO_4 (Li et al., 1998). One of the methods proposed in literature, which leads to the preparation of plane and good quality membrane, is the anodisation in two steps. This method was proposed for the first time by Masuda and Fukuda (Masuda & Fukuda, 1995). The characteristics of the prepared alumina membrane depend on the anodisation conditions (the concentration of the electrolyte used to modify the anodisation conditions, the working temperature, the anodisation potential). Thus, by the modification of anodisation conditions alumina membrane with pores with diameters between 20 nm and 400 nm can be obtained. The alumina nanoporous membrane can be also obtained by the combination of anodisation process and nanoindentation process. This technique consists in the creation of an array of defects on the aluminium surface which will serve as nucleation centre for pores in the next anodisation step. The nanoindentation technique allows the preparation of nanoporous membranes with ordered pores by one step anodisation process. In this case the distance between pores and the membrane porosity can be controlled as well. It is worth to be mentioned the fact that by using this technique, arrays of pores with different symmetries can be prepared (Masuda et al., 2001; Asoh et al., 2001; Vojkuvka et al., 2008).

The nanowires made up of noble metals and transition metals are the most important types of studied nanowires due to their versatility in applications such as biosensors or magnetic elements. In function of the application field the nanowires' properties can be studied either in the membrane or "free", after the dissolution of the membrane.

The magnetic nanowires represent a class of nanosized materials in the shape of nanowires intensively studied in the last years is. This family of nanowires is interesting because of their magnetical and transport properties (giant magnetoresistance, reversal magnetization in only one nanowire) being of significant interest due to their potential to work as sensing elements in chemical biological sensors or in optical and electronic devices. The special properties of nanowires can be used in various applications (spintronics, miniaturization of magnetic sensors, ultrahigh-density magnetic storage media, etc.).

The interesting physical properties of magnetic nanowires reside in their geometry and in their dimensionality. The studies presented in the literature on simple magnetic nanowires based on Fe, Co and Ni show that the magnetic properties of nanowires materials are different from the bulk material. This is especially related to the shape anisotropy (Nielsch et al., 2001; Sarkar et al., 2007; Nguyen et al., 2006). The research studies show at the same time that the magnetic properties of nanowires are function of the pH value of the preparation solution. For instance, depending on the pH value of the solution, the cobalt nanowires present two different crystallographic structures: hexagonal or cubic. Thus, the cobalt nanowires prepared at pH 3 have a cubic structure while the nanowires prepared at a pH ranging between 3,5 and 6 present a hexagonal structure (Li et al., 2004, Encinas et al., 2002; Ren et al., 2009, Sanchez-Barriga et al., 2007) which confer different magnetic properties to the nanowires synthesized from different pH solutions.

The giant magnetoresistance (GMR) studies of magnetic nanowire arrays started in the nineties (Piroux et al., 1994) and is continuing nowadays (Nasirpour et al., 2007; Huang et al., 2009). The GMR effect is observed in magnetic multilayered nanowires when the ferromagnetic elements are layered with nonmagnetic elements (Figure 1).

Fig. 1. Sketch of multilayered magnetic nanowires with GMR effect

The advantage of the use of multilayer nanowires (especially of NiFe/Cu magnetic nanowires) was intensively studied. The magnetic and magnetoresistance properties of this type of nanowires depend on the NiFe and Cu layers thickness (Chiriac et al., 2009).

The noble metal nanowires sequentially deposited (multilayered nanowires such as: Au/Pt, Au/Ag, or Ag/Pt) can be used as "bar-codes" in biological testing (Nicewarner-Peña et al.,

2001). Thus, sequences of different metals in a single nanowire adsorb different molecules which can be used to simultaneously detect different biological molecules.

Further on, a conventional method of synthesis of gold, silver and platinum simple nanowires and gold/platinum multilayer nanowires by electrochemical deposition will be described. In this work we used a VOLTALAB 10 PGZ 100 potentiostat in order to control the applied voltage during the electrodeposition. After electrodeposition is complete, the AAO template filled with noble metals were characterized by scanning electron microscopy (SEM) by using a JEOL microscope equipped with energy dispersive X-ray spectroscopy (EDS) analysis tool and by current-atomic force microscopy (I - AFM) using a Park microscope.

2. Experimental

The nanowires were growth inside an anodic aluminum oxide (AAO) template provided by Whatman. This template has a specific pore size of 200 nm and a thickness of 50 μm . For performing the electrochemical deposition, we used a three- electrode cell: as reference we used SCE for gold and platinum electrodeposition and, in order to avoid the precipitation of the silver chloride during the electrodeposition, we used a graphite electrode for silver electrodeposition. For all the experiments, as counter electrode we used Pt foil. Prior to electrodeposition, an adhesion layer of Au film was spread onto one side of the AAO template by thermal evaporation in order to cover the pores completely, and to serve as the working electrode during electrochemical deposition. All the experiments were performed at room temperature. The electrodeposition experiments were performed by pulsed electrodeposition (Inguanta, 2009). Platinum nanowires were growth in aqueous solution of H_2PtCl_6 5 mM/L and HCl 0.1M by applying a dc current of -0.2 V for 3 s and 0 V for 1 s. In the case of gold nanowires deposition we have used an aqueous solution of HAuCl_4 5 mM/L and H_3BO_3 0.5 M. The electrodeposition was performed by applying a dc current of -1.3 V for 5 s and 0 V for 1 s. The silver nanowires were deposited from an aqueous solution of AgNO_3 30 g/L and H_3BO_3 45 g/L at -0.7 V for 5 s and 0 V for 1 s. The electrodeposition potential was determined by linear voltammetry.

3. Results and discussions

After electrodeposition was carried out, the cross section of the AAO template filled with noble metals were characterized by scanning electron microscopy (SEM) using a JEOL microscope. Figure 2 shows the SEM micrographs of the cross sections of the AAO: filled with platinum nanowires (Figure 2a), filled with gold nanowires (Figure 2b), filled with silver nanowires (Figure 2c).

The image analysis show that the membranes are homogeneous filled with nobles metals. The growth rate of metals nanowires is changing in function of the nature of the electrodeposited metals: for platinum deposition, the growth rate - 2 $\mu\text{m}/\text{h}$, for gold - 18 $\mu\text{m}/\text{h}$ and for silver - 11 $\mu\text{m}/\text{h}$. After the deposition, the AAO template was dissolved by immersing it in a KOH 5M solution in order to liberate the noble metals nanowires. After the dissolution of the template, the nobles metal nanowires are rinsed several times with distilled water in order to remove the potassium hydroxide from the nanowires surface. In Figure 3 are presented the SEM images of the noble metals nanowires free of the alumina template.

(a)

(b)

(c)

Fig. 2. Cross section SEM micrographs of a AAO template filled with platinum nanowires (a), gold nanowires (b), silver nanowires (c)

(a)

(b)

(c)

Fig. 3. SEM micrographs of noble metals nanowires liberate from the AAO template: platinum nanowires (a), gold nanowires (b), silver nanowires (c).

The freed nanowires were collected from the hydroxide solution via centrifugation and rinsed several times with distilled water. Thereafter, the nanowires were submitted to EDS analysis. The EDS spectra (Figure 4) show that the obtained nanowires do not contain impurities (the detected elements are platinum, gold, silver and titanium). The titanium tracks present in all the spectra showed in Figure 4 comes from the sample holder whereas the gold comes from the thin layer deposited by thermal evaporation in vacuum which ensured the electrical conductivity.

Fig. 4. EDS analysis of platinum nanowires (a), gold nanowires (b), silver nanowires (c)

The obtained noble metals nanowires are individually characterized by current atomic force microscopy (I – AFM). For performing an accurate analysis the surfaces must be very smooth. Therefore, after electrodeposition, the electrodeposited alumina samples are submitted to a mechanical polishing process by using diamond (particles size – 3 μm) and Syton (particles size – 20 nm). The role of this step is to bring the nanowires to the same length on the surface and to obtain very smooth surfaces. After each polishing step, the AAO surface is visualized with the SEM microscope. Figure 4 shows the top-view SEM micrograph of the mechanically polished alumina membrane filled with platinum (Figure 5a), gold (Figure 5b), and silver (Figure 5c).

Fig. 5. Top-view SEM micrograph of mechanically polished alumina membrane filled with platinum nanowires (a), gold nanowires (b), silver nanowires (c).

This top-view SEM micrograph shows that noble metal nanowires are of the same diameter and shape as the alumina membrane pores. When the polishing process is finished, the surface of the AAO template filled with nanowires is examined by current-atomic force microscopy (I - AFM) by applying a +1 V dc bias current between the AFM tip and the sample's surface. In I-AFM mode, a conductive AFM tip scans the surface while it is in contact. This technique is able to image simultaneously both the topography and the conductivity of the surface. The current flowing between the tip and the sample gives us information about the surface conductivity of the sample. Contact topography image is generated by using a feedback loop to maintain the constant tip deflection whereas the I-AFM image is generated by measuring the current flow. In Figure 6 is presented the topographically and the electrically images of mechanically polished alumina membrane filled with silver nanowires.

Fig. 6. I – AFM images of mechanically polished alumina membrane filled with silver nanowires; the topography is shown in (a), and the simultaneously recorded (at +1 V bias voltage) surface conductivity in (b)

The topographical image of the nanowires correlates very well with the peaks on the current map. Close to 100% of nanowires were found to be conductive.

Nanowire functionalization

The functionalization of nanowires with (bio)molecules represents a chemical process in which a strong covalent bond is formed between the nanowire and the (bio)molecules (Mbindyo et al., 2001).

The functionalization of metallic nanowires with biomolecules represents one of the recent applications of nanomaterials. The unique physical properties of nanomaterials to recognise biomolecules in a selective mode can lead to the miniaturisation of biological sensors.

Although the use of nanowires in biosensors is of high interest, the electrochemical synthesis of simple and multilayered nanowires which contain noble metals is difficult, the influence of the electrochemical deposition parameters not being very well known.

There are a significant number of methods based on chemical approaches for surface functionalization. Well-documented collections of bioconjugation and functionalization techniques are available now. Bioconjugation involves the linking of two or more molecules to form a new complex having the combined properties of its individual components (Hermanson, 2008). As a straightforward example, we recommend for a detailed analysis the Hermanson's collection of methods (Hermanson, 2008) that can be used for a lot of functionalization processes.

In the case of metallic nanowires, the functionalization with different organic natural or synthetic molecules follows commonly the same way as for their bulk counterparts. However, high differences occur when the specific magnetic, optic, electric etc. properties are investigated and compared.

As a basic rule, the functionalization of the metallic nanowires have to take into account the chemical affinity between the metal surfaces and the (bio)molecules used. It was experimentally observed that the chemical properties of the surfaces play a crucial role in the binding process, the chemical groups imposing variations in reactivity for different metallic surface (table 1).

Ligand	Name	Surface for modification	Proposed linkage
R-SH R-S-S-R'	Thiols Disulfides	Au, Ag, Cu, Hg, Fe	R-S-Surface
R-CN	Cyanides	Pt, Pd	R-CN-Surface
R-(CO)-OH	Carboxilic Acids	Metal oxides	R-(CO)-Surface
R-(PO ₂)-OH	Phosphonates	Metal oxides	R-(PO ₂)-Surface
R ₂ -Si-O-R	Siloxanes	Metal oxides	R ₂ -Si-O-Surface
R-(CO)-NH-OH	Hydroxamic acids	Metal oxides	Surface-O-(CR)-(NH)-O-Surface

Table 1. The most used chemical groups for surface functionalization of different metals (Reich et al., 2011)

Here, we are focusing mainly on the functionalization of gold-based nanowires as representative ones for the potential applications in the biomedical field because gold nanomaterials have proven to be versatile biomedical tools due to their particular structural and physico-chemical properties (Ray et al., 2011).

For functionalization of gold nanowires, a preferred method is the self-assembling of (bio)molecule monolayers. For instance, a mercaptoundecanoic acid (HS(CH₂)₁₀CO₂H) was used to coat Au-based nanowires resulting in surfaces functionalized with carboxylate groups that allowed the use of carbodiimide chemistry to conjugate primary amine groups of a capture antibody to the carboxylate groups on the nanowires. (Tok et al., 2006; Hermanson, 2008).

Single-strand DNA can be also specifically modified in order to meet the affinity requirements of a metallic surface for coupling. For example, in the case of a gold nanowire, a thiol group was inserted in the 5' position of a single-strand DNA whereas tetramethyl rhodamine was introduced in the 3' position. The optical images showed the single-strand DNA reacted with gold nanowires through the thiol groups (Mbindyo et al., 2001).

At the surface level, multi-segment nanowires can make available a diversity of chemical properties that can be used to selectively functionalize the metallic segments. Because the metallic stripes present different chemical reactivity and, therefore, reacts differently towards the chemical groups of the biomolecules, a selective functionalization can be carried out in function of the metals making up the nanowire. For example, a Au-Pt-Au nanowire can be functionalized both with thiols and isocyanides. Due to affinity of thiols towards Au, a self-assembled-monolayer of 2-mercaptoethylamine can be formed on the gold surface whereas a butaneisonitrile monolayer attaches to the Pt segments (Kovtyukhova et al., 2002). The biomolecules can be modified with fluorescent markers in order to spatially discriminate the position of the biomolecules along the stripped nanowires.

Gold nanowires with additions of magnetic materials, such as nickel, can be also selectively functionalized. For example, Au surface can be functionalized with thiol-based hexa(ethylene glycol) groups whereas Ni surfaces were functionalized with palmitic acid. Fluorescently-marked proteins bound to hydrophobic palmitic acid lead to a bright fluorescence whereas thiol-based hexa(ethylene glycol) groups do not allow the protein to attach to it (Birenbaum et al., 2003). A secondary role of the nickel segments is to endow the nanowires with magnetic properties.

For certain conditions, gold nanostructures present unexpectedly some ferromagnetic or paramagnetic-like properties. Thus, it has been shown that gold nanostructures capped with alkanethiols present an important ferromagnetic behaviour as compared with their non-functionalized counterparts. For instance, the simultaneous presence of Au-Au and Au-S bonds, conjugated with the creation of an ordered self-assembled monolayer shell, is considered key parameters for the ferromagnetic-like behaviour revealed by the thiol-functionalized gold nanostructures. The magnetic properties become obvious and manifest when the capping organic molecules form self-assembled monolayers on gold substrates. It was experimentally established that the simultaneous presence of Au-Au and Au-S bonds is required to observe ferromagnetic behaviour in thiol-functionalized nanostructures. Polymeric-like phases (-Au-S-Au-S- bonds) do not show magnetization properties. (Guerrero et al., 2008).

Multi-component nanowires based on three Ni/Au/Ni segments can be also fabricated and functionalized. The preparation of multilayered nanowires allows the selective functionalization of different segments since the metallic segments present different chemical characteristics (Kovtyukhova & Mallouk, 2002). For example, a thiol-modified single strand DNA and a biotinylated peptide were tailored to selectively attach to the gold and nickel segments, respectively, by which a F1-ATPase motor can be bound only to the nickel segment of the nanowires by using the biotin-streptavidin linkage. Also, the gold segments of nanowires were functionalized by using fluorescent single strand DNA molecules. The process is based on the strongly binding between thiol groups on the single strand DNA and the gold surface. Also, in order to be optically detected the nickel segments of nanowires were functionalized by using fluorescent biotinylated peptide. (Ren et al., 2006).

Nanowire-based detection of disease-specific DNA

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 such as Polymerase Chain Reaction (PCR), Restriction Fragment Length Polymorphism (RFLP), Real Time - Polymerase Chain Reaction (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, we tested the ability of the naked gold-based nanowires to be used in a biodetection assay for

identification of a DNA sequence, specific for FLT3 gene mutation, responsible for acute myeloblastic leukemia.

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

1. Separation and purification of specific genomic DNA from the blood of patients with acute myeloblastic leukemia, in order to detect mutation of FLT3 gene;
2. Amplification of target DNA sequence through PCR 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 on the surface of the metallic nanowires and further detection through a 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 (Miller et al., 1988; Beutler et al., 1990). Second, amplification of the 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 by measuring the fluorescence generated by the fluorophore-tagged amplicons (i.e. products resulted from PCR amplification process) immobilized on nanowires (figure 7).

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 (figure 7).

Fig. 7. (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 figure 7(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

laminae of the microscope, but in small groups. This behaviour is due to the typical physical forces governing interactions in liquids. However, the image shows the successful immobilization and qualitative detection of DNA, specific to FLT3 gene involved in acute myeloblastic leukemia.

However, in spite of the successful qualitative detection, based on a comparative study, 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. Conclusion

In synthesis, by using a conventional electrodeposition process, the arrays of single Pt, Au and Ag nanowires have successfully been fabricated by pulsed electrodeposition. The obtained nanowires have been investigated by SEM and I - AFM. The results showed that the obtained nanowires have a diameter of about 200 nm and a length of several micrometers. All the samples have been mechanically polished and we have showed that the AAO membranes are fully filled with metallic compound. The I - AFM microscopy have showed that the as-obtained nanowires are continuous inside the membrane.

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 be carried out.

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Noble Metals

Edited by Dr. Yen-Hsun Su

ISBN 978-953-307-898-4

Hard cover, 426 pages

Publisher InTech

Published online 01, February, 2012

Published in print edition February, 2012

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How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Dragos-Pinzaru Oana-Georgiana, Dumitru-Daniel Herea and Horia Chiriac (2012). Synthesis and Characterization of Noble Metal Nanowires, Noble Metals, Dr. Yen-Hsun Su (Ed.), ISBN: 978-953-307-898-4, InTech, Available from: <http://www.intechopen.com/books/noble-metals/synthesis-and-characterization-of-nobles-metals-nanowires>

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