Electrochemistry Communications 63 (2016) 18–21

Contents lists available at ScienceDirect

Electrochemistry Communications

journal homepage: www.elsevier.com/locate/elecom

Short communication

Electrochemical preparation and characterization of magnetic core–shell nanowires for biomedical applications

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ARTICLE INFO

Article history:
Received 23 October 2015
Received in revised form 4 December 2015
Accepted 8 December 2015
Available online 15 December 2015

Keywords:
Electrochemical synthesis
Core@shell magnetic nanorods
Functionalization
Cellular viability

ABSTRACT

Magnetic CoNi@Au core–shell nanorods have been electrochemically synthesized, characterized and functionalized to test their inherent cytotoxicity in order to assess their potential use for biomedical applications. The initially electrodeposited CoNi nanorods have been covered with a gold layer by means of galvanic displacement to minimize the nanowires toxicity and their aggregation, and favour the functionalization. The presence of a gold layer on the nanorod surface slightly modifies the magnetic behaviour of the as-deposited nanorods, maintaining their soft-magnetic behaviour and high magnetization of saturation. The complete covering of the nanorods with the gold shell favours a good functionalization with a layer of (11-Mercaptoundecyl)hexa(ethylene glycol) molecules, in order to create a hydrophilic coating to avoid the aggregation of nanorods, keeping them in suspension and give them stability in biological media. The presence of the organic layer incorporated was detected by means of electrochemical probe experiments. A cytotoxicity test of functionalized core–shell nanorods, carried out with adherent HeLa cells, showed that cell viability was higher than 80% for amounts of nanorods up to 10 μg mL^{-1}. These results make functionalized nanorods promising vehicles for targeted drug delivery in medicine, which gives a complementary property to the magnetic nanoparticles.

1. Introduction

Electrochemical methods have been recently demonstrated as very useful for the synthesis of metallic nanostructures as nanoparticles, nanotubes or nanorods [1–2], which have been applied in catalysis [3], sensing [4] or computer science [5]. On the other hand, magnetic nanoparticles, especially of iron oxides, are being used in biomedicine for treatment as hyperthermia or drug delivery [6–9]. Recently, the possibility of using nickel nanowires in biomedicine has been also proposed [10–12]. In the present work we propose the test of CoNi@Au nanorods (NRs), of a few microns of length, as magnetic supports that could be functionalized and show little cellular toxicity.

CoNi NRs present high magnetization of saturation and chemical stability, being good magnetic vehicles for the transport of therapeutic molecules. Moreover, the shape of these nanostructures can present the advantage, respect to nanoparticles, of improving the therapeutic molecules/vehicle ratio. However, structures of less than 5 μm and nanometric diameter will be prepared, in order to permit their circulation by the blood vessels in possible medical applications. In order to favour both the biocompatibility and the functionality of the nanorods, a complete thin layer of gold is induced on the surface, being its correct formation electrochemically tested. The core@shell NRs are functionalized with (11-Mercaptoundecyl)hexa(ethylene glycol) (MHEG) to make hydrophilic their surface, minimize the aggregation of the magnetic nanorods and favour the interaction with the cells present in the culture. The viability of HeLa cells in the presence of the CoNi@Au-MHEG NRs will be analysed.

2. Experimental

Nanorod preparation and electrochemical tests were carried out at room temperature using a potentiostat/galvanostat Autolab PGSTAT30 (GPES software), and a cell with three electrodes, being a Ag/AgCl/KCl 3M the reference and a platinum spiral the auxiliary electrode.

The nanorods were prepared in Millipore polycarbonate membranes (20 μm-thick, 100 nm nominal pore size diameter), metalized by vacuum evaporation (in IMB-CNM.CSIC of Barcelona) with gold (100 nm-thick) on one side, using a $CoCl_2$ 0.2 M + $NiCl_2$ 0.9 M + H_3BO_3 0.5 M, pH = 3 solution. The synthesized nanorods were exhaustively cleaned in chloroform. The gold layer formation was carried out by means galvanic displacement, by immersing the nanorods in a $HAuCl_4$ 2.9 mM

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http://dx.doi.org/10.1016/j.elecom.2015.12.006
1388-2481/

solution at different times. The complete formation of the gold shell was tested by placing some nanorods in a vitreous carbon electrode (2 mm diameter) and testing their voltammetric response in a H_2SO_4 0.5 M solution. In all cases, argon flow was used before each experiment, maintaining argon atmosphere during the study. The solutions were always prepared with Millipore MilliQ water (18.2 MΩ·cm^{-1}).

The nanorods were examined by means of a Field Emission Scanning Electron Microscopy FE-SEM (JSM-7100F). An X-ray analyser (EDS) incorporated in a Leica Stereo Scan S-360 Equipment was used to determine the elemental composition. The magnetic behaviour was analysed by means of a SQUID magnetometer at 300 K.

To improve the solubility and biocompatibility of the nanorods, 150 μg of nanorods was functionalized with 1 mL of 8 mM MHEG solution in ethanol overnight, with shaking at room temperature. The nanorods were then washed with equal volume of ethanol 3 times with sonication for 5 s. The presence of MHEG molecules on the CoNi@Au NRs has been corroborated by means of electrochemical probe experiments, by recording cyclic voltammetries of the Fe(II)/Fe(III) system in a KNO_3 0.2 M + 2 mM $K_4[Fe(CN)_6]$ + 2 mM $K_3[Fe(CN)_6]$ solution in CoNi@Au and in CoNi@Au-MHEG NRs. For each experiment, 2.5 μL of a NR suspension (15 μg in 1 mL of a water–ethanol mixture) was dropped on the surface of a glassy carbon (GC) rod (0.031 cm^2 of diameter) and dried under nitrogen.

Human HeLa cervical adenocarcinoma cells (ATCC CCL-2) were used to test the cytotoxicity of nanorods. HeLa cells were cultured at 37 °C in a humidified sterile atmosphere of 95% air and 5% CO_2, using complete growth medium (DMEM) supplemented with foetal bovine serum (10% *v*/*v*), glucose (4.5 g L^{-1}), L-glutamine (292 mg L^{-1}), streptomycin sulfate (50 mg L^{-1}) and potassium penicillin (50,000 U L^{-1}). All the media, sera and antibiotics were provided by Gibco-Life Technologies (Paisley, UK). Cells were seeded in 96-well plates (3300 cells well^{-1}, Luna Automated Cell Counter) and grown up to 70–85% confluence. Cells were then incubated with different concentrations of CoNi@Au-MHEG nanorods (from 0.003 to 52 μg mL^{-1}) for 24 h. Cells incubated with the complete medium in the absence of nanorods were used as control. After incubation, the medium was discarded, cells were washed three times with cold PBS and incubation was followed for 24 additional hours with fresh culture medium. Cell viability was determined by means of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide (MTT, Sigma-Aldrich) assay. Briefly, the remaining HeLa cells were incubated with 0.05 mg MTT mL^{-1} in complete DMEM for 3 h. The medium was discarded, formazan crystals were solubilized with pure DMSO and formazan concentration was determined by absorption at λ_{ex} of 526 nm (microplate reader SynergyMx™, BioTek Instruments, Inc.). Cell viability was determined by the ratio between the absorbance of treated cells and that of non-treated cells (control, 100% viability).

3. Results and discussion

The protocol for the nanorod preparation was the following: 1) determination of the mass of the membrane, 2) immersion of the membrane in water during 24 h, 3) electrodeposition of the CoNi NRs in the described solution at −1.0 V and deposition charge of 4.4 C for a geometrical area of 3.1 cm^2 and a porosity of the 13.8%, 4) determination of the mass of the membrane containing the nanorods, 5) removing of the gold layer of the membrane with a saturated KI_3 solution, 6) removing of the membrane and cleaning (10×) of the nanorods with chloroform. Good reproducibility was obtained in successive synthesis, showing the Fig. 1a a representative chronoamperometric curve of the electrodeposition. The shape of the curve reveals the initial nucleation and growth of the CoNi on the gold in the bottom of the membrane's channels, the posterior decay due to the depletion of the electroactive species near of the growing nanorods and a final stabilization of the current when a stationary regime of transport of the electroactive species in the interior of the channels is attained.

The SEM pictures of the as-deposited nanorods show their dimensions and good definition. CoNi NRs of 65 wt.% of Co, 110 ± 15 nm of real diameter, 3.7 ± 0.4 μm and around 34 of aspect ratio were obtained

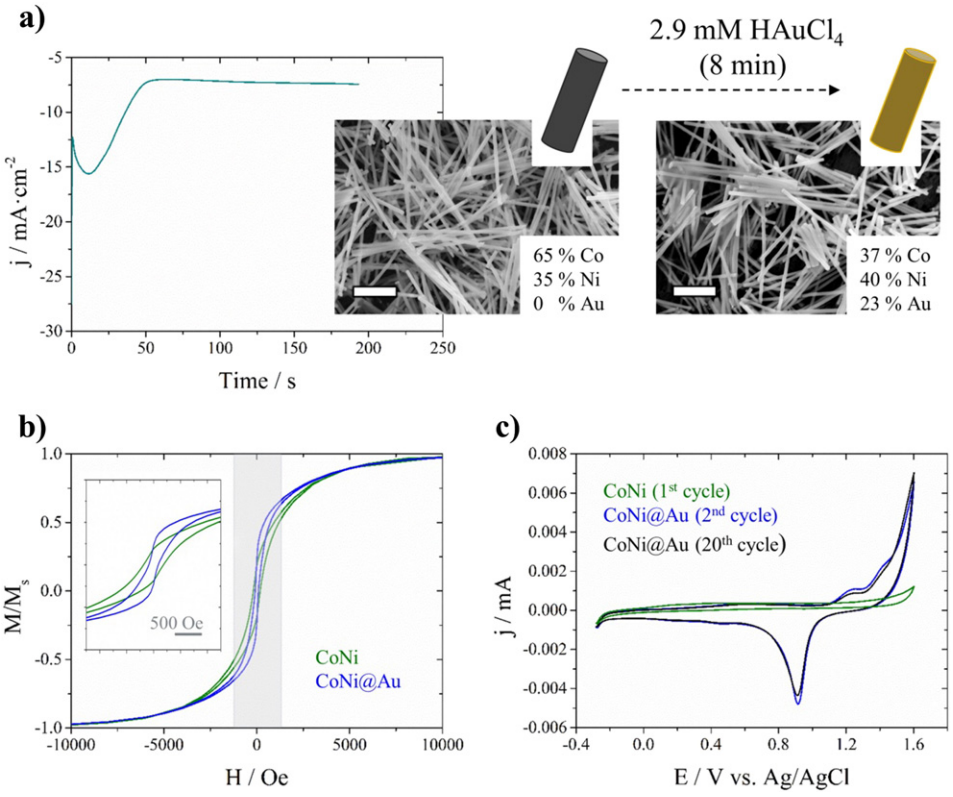

Fig. 1. (a) Representative chronoamperometric curve and FE-SEM pictures of CoNi and CoNi@Au NRs; scale bar: 1 μm. (b) Magnetic behaviour of the two types of NRs and (c) cyclic voltammetry at 100 mV s^{-1} of the nanorods in a H_2SO_4 0.5 M solution.