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Cytotoxicity of noble metal nanoparticles sputtered into glycerol

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ABSTRACT

Nowadays, nanomaterials belong among one of the most studies materials. Even despite the immense advantages of nanostructured materials, some studies indicate that these materials can also harmfully affect both human being and the environment. Therefore, in this study, we have focused on nanoparticle (NP) cytotoxicity in vitro. To study NPs intrinsic biological properties, we employed cathodic sputtering into glycerol for their synthesis, thus eliminating the use of toxic solvents. According to the transmission electron microscopy and dynamic light scattering measurements, we prepared non-agglomerated NPs of the following sizes: 6.1 ± 1.0 nm for gold, 4.2 ± 0.9 nm for silver, 2.5 ± 0.6 nm for palladium, and 1.8 ± 0.4 nm for platinum NPs. The cytotoxic activity of these NPs was determined by WST-1 assay using six model cell lines: human cells from hepatocarcinoma (Hep G2), human keratinocytes (HaCaT), mouse macrophages (RAW 264.7), mouse embryonic fibroblasts (L929 and NIH 3T3), and cells from Chinese hamster ovary (CHO-K1).

Introduction

Nanomaterials have been recently applied in wide variety of technical fields covering electronics, medicine, cosmetics, etc. A nanomaterial is defined as such substance which has a size between 1 and 100 nm in at least one of its dimensions. Such material characteristics result in absolutely unique physico-chemical properties, e.g. different thermal conductivity [1], photocatalytic activity [2] and electric properties [3], which are hardly predictable and they largerly differ from the bulk of the material. Even despite tremendously advantageous properties, which exhibits these nano-sized materials, there occurs studies suggesting that these man-made entities have a negative impact on both human beings and the environment. Nevertheless, there is still a lack of complex knowledge concerning this issue. Therefore, it is crucial to explore these potential risks connected with nanomaterials in order to ensure their safer handling and applications [4].

When assessing biological properties of nanostructured materials, it is necessary to take into account the way of their synthesis. The most frequently used chemical preparations, so called wet, often involve a use of toxic solvents, and thus they are not relevant for biological properties assessment [5]. That is why it is necessary to search for novel ways of nanoparticle (NP) preparations, which will eliminate the use of toxic agents. On top of that, it will yield limited size distribution of the arisen particles. Cathodic sputtering into liquids [6] and other biological-based techniques [7] belong among these methods.

The level of NP cytotoxicity is dependent mainly on the following factors: (i) chemical composition, (ii) size, (iii) shape, and (iv) concentration [8]. The most likely explanation of NP cytotoxic mechanism for living eukaryotic cells has been described for silver NP. In the first step the NP is recognized by surface membrane receptors, then it is incorporated into the plasma membrane, which is followed by translocation into the intracellular space. Inside the cell, NPs accumulate in the organelles, which are subsequently degraded. The most often affected ones are endosomes and lysosomes. The enormous amount of the NPs inside the cell results in a number of adverse effects, such as oxidative stress, cell membrane and DNA damage, cell cycle arrest, apoptosis and/or genotoxicity [9].

In this work we attempt to assess the cytotoxic effect of noble metal NPs towards selected cell lines with respect to specific NPs concentrations.

2. Experimental

2.1. Materials, apparatus and procedures

Metal deposition was performed by SputterCoater SCD 050 (BAL-TEC) using 99.999% pure gold, silver, platinum and palladium

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targets. Sputtering was accomplished at room temperature (20 °C), deposition time of 300 s, current of 40 mA, voltage of 420–430 V, total argon pressure of 4–6 Pa (gas purity 99.99%), an electrode distance of 5 cm.

As a capturing media for preparation of metal NPs we used anhydrous glycerol (propane-1,2,3-triol, Penta, Mw = 92.1 g mol^{-1}, purity 99.8%). The Petri dish of inner diameter of 4 cm was filled with 5 ml of glycerol. After the metal sputtering, the glycerol with metal NPs was transferred into 40 ml vials and mixed with distilled water in the volume ratio of 1:3 (glycerol:water). More detailed description of the deposition procedure can be found in Ref. [10].

2.2. Analytical methods

Prepared solutions of colloidal metal NPs have been characterized by transmission electron microscopy (TEM), dynamic light scattering (DLS) and atomic absorption spectrometry (AAS). Furthermore, their cytotoxic activity was determined.

Samples for TEM were centrifuged and NPs transferred into distilled water. Drop of colloidal solution was placed on a copper grid coated with a thin amorphous carbon film on a filter paper. The excess of solvent was removed. Samples were air dried and kept under vacuum in a desiccator before placing on a specimen holder. TEM characterization of the sample was performed using JEOL JEM-1010 (JEOL Ltd., Japan) operated at 400 kV. Particle size was measured from the TEM micrographs and calculated by taking into account at least 500 particles.

The particle size was also determined by Zetasizer ZS90 (Malvern Instruments Ltd., England) in the DLS regime for particle size distribution equipped with an avalanche photodiode for detecting the signal. Diode pumped solid state laser (50 mW, 532 nm) was used as a light source. The measurements were performed in polystyrene cuvettes at room temperature.

Concentrations of prepared nanoparticles were determined by means of AAS by a VarianAA880 device (Varian Inc., USA) using a flame atomizer at 242.8 nm wavelength. Typical uncertainty of concentration determined by this method is less than 0.5%.

2.3. Cytotoxicity tests

Cytotoxicity of Ag, Au, Pd, and Pt NPs was assessed by WST-1 assay (Roche, Germany) based on tetrazolium salt (WST-1) reduction resulting in soluble formazan by the mitochondrial oxidoreductases in metabolically active cells. Formed formazan was measured spectrophotometrically at 450 nm (reference wavelength 630 nm) using UV–vis spectrometer (BioRad). The absorbance is directly proportional to the amount of arisen formazan, which is proportional to the number of metabolically active cells.

HaCaT (human keratinocytes), Hep G2 (human cells from hepatocarcinoma), CHO-K1 (Chinese hamster ovary cells), RAW 264.7 (mouse macrophages), NIH 3T3, and L929 (mouse embryonic fibroblasts) cell lines were seeded into individual wells of 96-well plates (5000–10,000 cells per well depending on the generation time of each cell line) in 100 μL of cell culture medium supplemented with 10% fetal bovine serum and 1% vitamins solution. The cells were incubated overnight (16 h) under standard cultivation conditions (37 °C, 5% CO_2, 95% humidity), then the culture medium was removed and replaced with 100 μL of fresh media with the tested NPs (the final NPs concentration was 0–6.15 mg ml^{-1}). NPs cytotoxicity was assessed after 24, 48 and 72 h of treatment by the following procedure: the medium was removed, the cells were incubated with 5 μL of WST-1 dissolved in 95 μL of complete medium without phenol red for 2 h, then the absorbance of formazan was measured. Cells incubated with medium only (without NPs) and cells incubated with the vehicle were used as controls. All experiments were done in quadruplicates.

3. Results and discussion

From the TEM images in Fig. 1, it is apparent that we successfully prepared spherical NPs of four noble metals. The individual sizes were following: 6.1 ± 1.0 nm for gold, 4.2 ± 0.9 nm for silver,

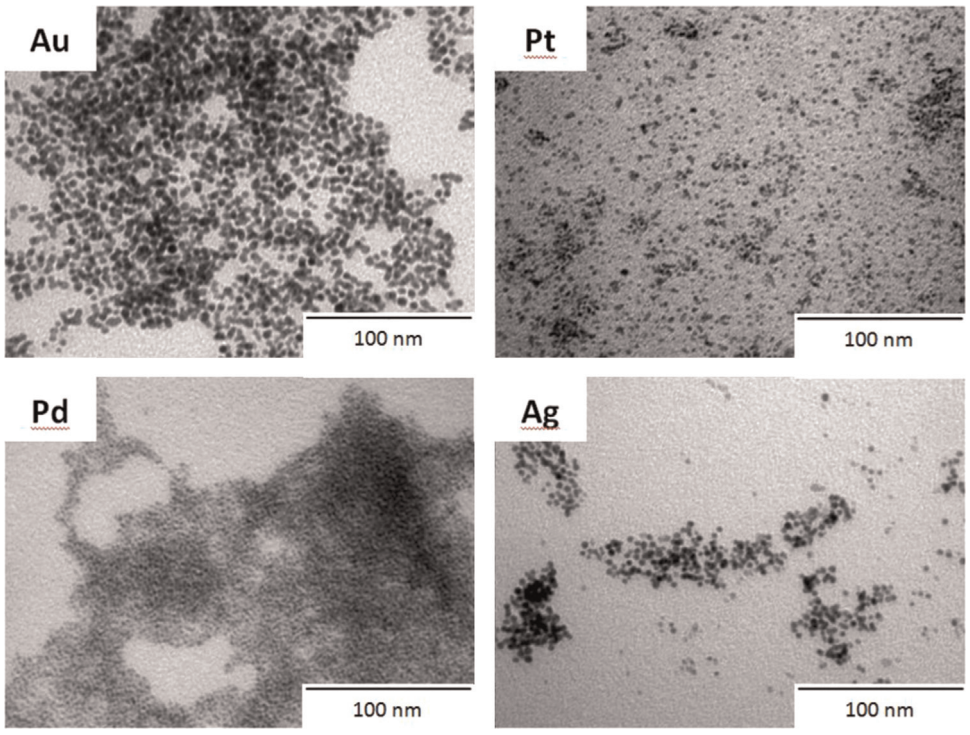

Fig. 1. TEM images of gold, silver, platinum and palladium nanoparticles.