METAL NANOPARTICLES PREPARED BY SPUTTERING INTO TEMPERRED GLYCEROL

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Abstract

Nowadays, nanoparticles (NPs) are enjoying unprecedented popularity due to their use in various applications. Even despite the enormous benefits of those man-made entities in numerous applications, many serious impacts on human health and the environment have been reported. To study NPs intrinsic biological properties, we chose a cathode sputtering for their preparation eliminating the use of toxic solvents and reducing agents. According to the transmission electron microscopy and dynamic light scattering measurements, we prepared non-agglomerated faceted NPs with diameter of 1.8 ± 0.4 nm and 2.3 ± 0.4 nm for platinum and palladium, respectively. We evaluated their cytotoxic activity by WST-1 assay using human embryonic kidney (HEK 293T) cell line. We also determined the antibacterial properties of these NPs against two common pollutants (*E. Coli*, a Gram-negative bacteria and *S. epidermidis*, a Gram-positive bacteria).We observed appreciable toxicity of metal NPs in all studied samples.

Keywords: Sputtering, metal nanoparticles, glycerol

1. INTRODUCTION

The production of metal nanoparticles (NPs) has drawn great attention of scientific and technological concern due to their applicability in various devices and processes, such as catalysis, biomedical applications, solid state devices etc. Their size and size-distribution is decisive in defining their unique properties compared to their bulk forms. Therefore the development of innovative methods for eco-friendly NPs production with control over their size and size-distribution is a major challenge for researchers all over the world.

NPs preparation by sputtering technique seems to be a promising method which meets the requirements for the modern nanoparticles synthesis. Sputtering into liquids completely excludes the use of undesirable toxic reducing agents and with appropriately chosen capturing media it ranks alongside environmentally friendly preparation methods. As a capturing media one can use a liquid with sufficient low vapour pressure and ability to stabilize the resulting nanoparticles. To date, the most commonly used capturing media are ionic liquids[1-4], vegetable oils[5, 6], polyethylene glycol [7, 8]. In 2013, Siegel et al. reported the usage of glycerol[9], which stands out for its biocompatibility. Combination of the cathode sputtering as a method and glycerol as a capturing media seems to be a promising configuration for subsequent evaluation of the biological properties of nanoparticles [10, 11].

The rate of NP cytotoxicity is generally determined by several factors: (i) size, (ii) shape, (iii) chemical composition, and (iv) concentration [12]. The most likely explanation of NP toxic mechanism for living eukaryotic cells has been described for silver NPs. 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 [13]

2. EXPERIMENTAL

2.1. Materials, apparatus and procedures

Metal deposition was performed by SputterCoater SCD050 (BalTec, Liechtenstein). Pure metal target (Pt, Pd - purity 99.999 %, Safina as., Czech Republic) was used for the deposition. Sputtering was accomplished at room temperature (20°C), deposition time of 300 s, current of 30 mA, voltage of 380 - 420 V, total argon pressure of 6·Pa (gas purity 99.99 %).

As a capturing media for preparation of silver NPs we used anhydrous glycerol (propane-1,2,3-triol, Penta, Czech Republic, Mw = 92.1 g·mol-1, purity 99.8 %). The Petri dish of inner diameter of 4 cm was filled with 3 ml of glycerol. After the metal sputtering, the colloidal solution was transferred into 40 ml vials and mixed with distilled water in the mass ratio of 1:3 (glycerol: water).

2.2. Analytical methods

Prepared solutions of metal NPs were characterized by dynamic light scattering (DLS) and transmission electron microscopy (TEM) and their biological activity were evaluated using drip test and WST-1 assay.

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 them on a specimen holder. TEM characterization of the samples 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.

2.3. Biological tests

2.3.1. Antimicrobial test

The antibacterial potency of Pd and PtNPs was assessed by drip method using two environmental bacterial strains, Gram-negative *Escherichia coli* (*E. coli*, DBM 3138) and Gram-positive *Staphylococcus epidermidis* (*S. epidermidis*, DBM 3179). The experiments were performed similarly as in work. The desired bacterial strains were cultured in nutrient broths (LB broth for *E. coli*, PCA broth for *S. epidermidis*) at 37°C overnight under continuous agitation. Optical densities of the overnight cultures were measured at 600 nm. The starter inocula were prepared by serial dilutions of the cultures in fresh sterile physiological saline solution (0.9 % w/v of NaCl). We examined the antimicrobial effects of these metal nanoparticles in dependence on bacterial concentration; *E. coli* was inoculated in density of $1 \cdot 10^3$, $1 \cdot 10^4$, $1 \cdot 10^5$, and $1 \cdot 10^6$ cells.ml^{-1}, *S. epidermidis* in density of $1 \cdot 10^4$, $1 \cdot 10^5$, $1 \cdot 10^6$, and $1 \cdot 10^7$ cells.ml^{-1}. Two microliters of freshly prepared PdNPs (11.8 mg.ml-1) and PtNPs (16.6 mg.ml^{-1}) were used per 1 ml of physiological saline solution. In parallel, *E. coli* and *S. epidermidis* incubated solely in physiological saline solution were used as controls. All samples were incubated statically at 24°C for 4 h, afterwards the samples were vortexed and 25 µl aliquots of each sample were dripped onto pre-dried LB (*E. coli*) and PCA agar (*S. epidermidis*) plates and incubated for 24 h at 24°C and 37°C for *E. coli* and *S. epidermidis*, respectively. The growth of both bacterial strains was evaluated by direct counting of the colony forming units (CFU). Samples were done in triplicates (plus 15 drops of each sample). The experiments were accomplished under sterile conditions.

2.3.2. Cytotoxicity test

Cytotoxicity of 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.

HEK 293T (human embryonic kidney) cell line was seeded into individual wells of 96-well plates (5,000 - 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 % CO2, 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

3.1. Size and size distribution

Prepared metal NPs are displayed on TEM images (**Figure 1**). From these images it is clear that we have successfully prepared nanoparticles with average diameter (1.8 ± 0.4) nm and (2.3 ± 0.4) nm in case of platinum and palladium, respectively. Additionally, we provided DLS measurement to determine average size and size-distribution of prepared NPs (see **Figure 2**). Observed size discrepancy is caused by intensity weighted mean particle diameter in case of DLS compared to number weighted diameter obtained by TEM[14]. Measured discrepancy is about 8% regardless of NPs type and is in a good agreement with published results[15]. More importantly, DLS proves that prepared NPs are non-agglomerated, which is of crucial importance for evaluation of their toxicity towards living organisms.

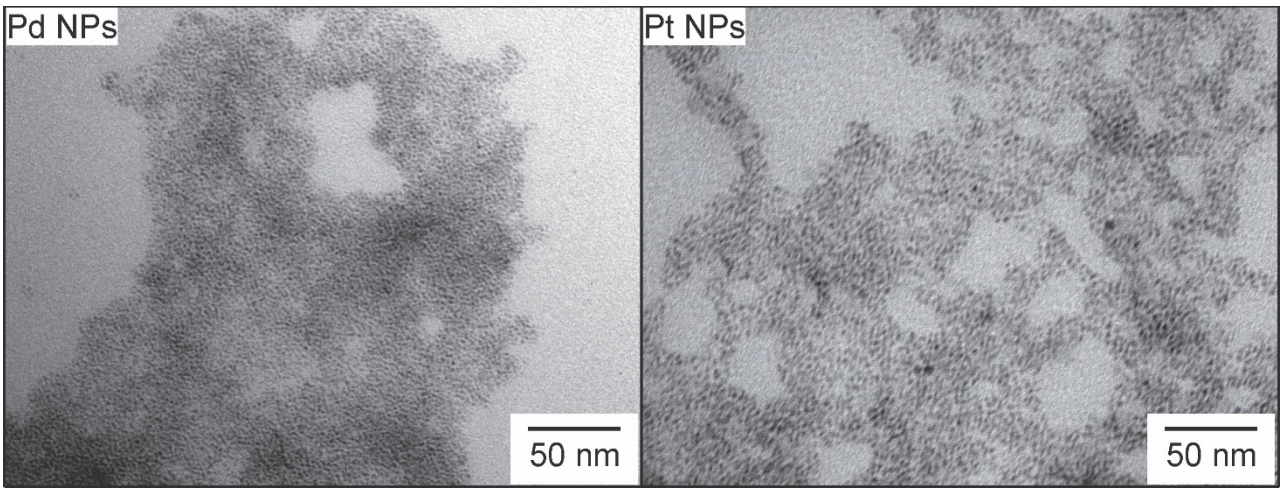

Figure 1 TEM images of Pd and PtNPs

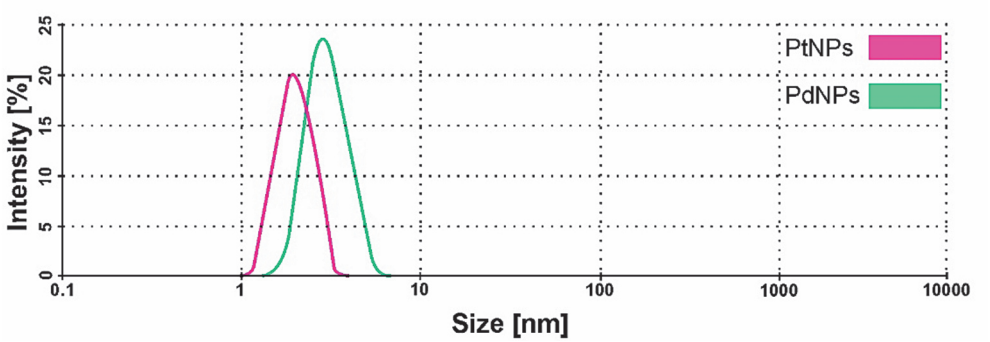

Figure 2 Characterization of aqueous solutions with platinum and palladium nanoparticles by dynamic light scattering

3.2. Antibacterial test

The inhibition activity of NPs against bacterial growth was evaluated by drip tests and the results can be seen in **Figure 3**. It is apparent that the incubation of *E. coli* with PdNPs had pronounced effect on its growth up to the concentration of $1 \cdot 10^5$ CFU per sample when compared to untreated control cells. This remarkable antibacterial activity of Pd diminished at higher bacterial concentrations starting from $1 \cdot 10^6$ CFU per sample. Similar potency of PdNPs was observed against *S. epidermidis*, even up to $1 \cdot 10^6$ CFU.

Because PtNPs exhibited very similar size as the palladium ones, we expected comparable antimicrobial potential. Nevertheless, we detected only insignificant inhibition of bacterial growth (*E. coli*) induced by PtNPs at concentration of $1 \cdot 10^5$ CFU when compared to the control samples. Surprisingly, we did not observe any growth inhibition of *S. epidermidis* in the presence of PtNPs. More importantly, we even recorded slight stimulation of their growth.

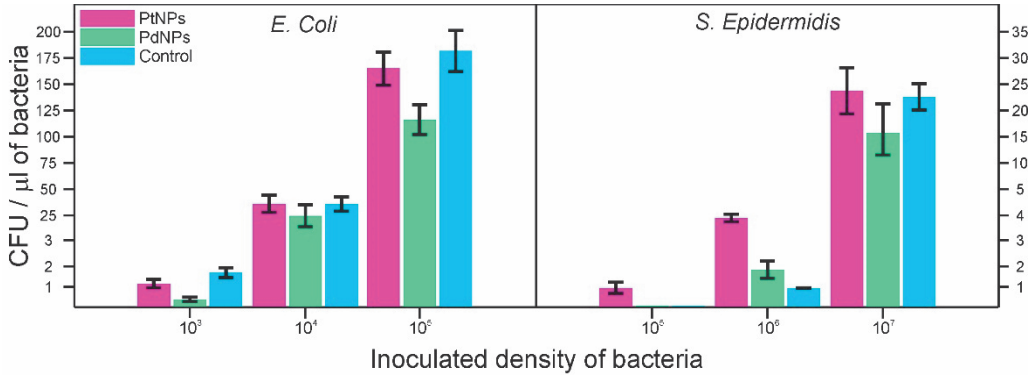

Figure 3 Inhibition effect of PtNPs and PdNPs towards bacterial strains of *E. coli* and *S. epidermidis* with different bacteria concentrations

3.3. Cytotoxicity

Even though nanoparticles of noble metals are of high commercial interest, information on their toxicology have been rather sparse. This knowledge could be advantageously employed in terms of their use in chemotherapeutical applications as novel candidates for e.g. tumor inhibitors. To assess the comparison study of *in vitro* cytotoxicity of Pd and PtNPs, we have used HEK 293T (human embryonic kidney) cell line, which is frequently used for toxicological assessments (for review see [16]). The cytotoxicity was determined in concentration (0 - 6,150 $\mu g \cdot ml^{-1}$) and treatment time (24, 48, and 72 h) dependent manner.

From **Figure 3** is obvious, that the cell viability was significantly reduced for NPs concentrations exceeding 1,000 $\mu g \cdot ml^{-1}$ at the first end point (after 24 h treatment), similar situation was observed after 48 and 72 h of

treatment. The results suggest that the final cytotoxicity is far more dependent on nanoparticle concentration, rather than on the treatment time. In comparison with recently published data [17-20], we observed much pronounced NPs cytotoxicity at corresponding concentration levels. This effect is most likely driven by the NPs size, which was much smaller in the case of our study (1.8-2.4 nm).

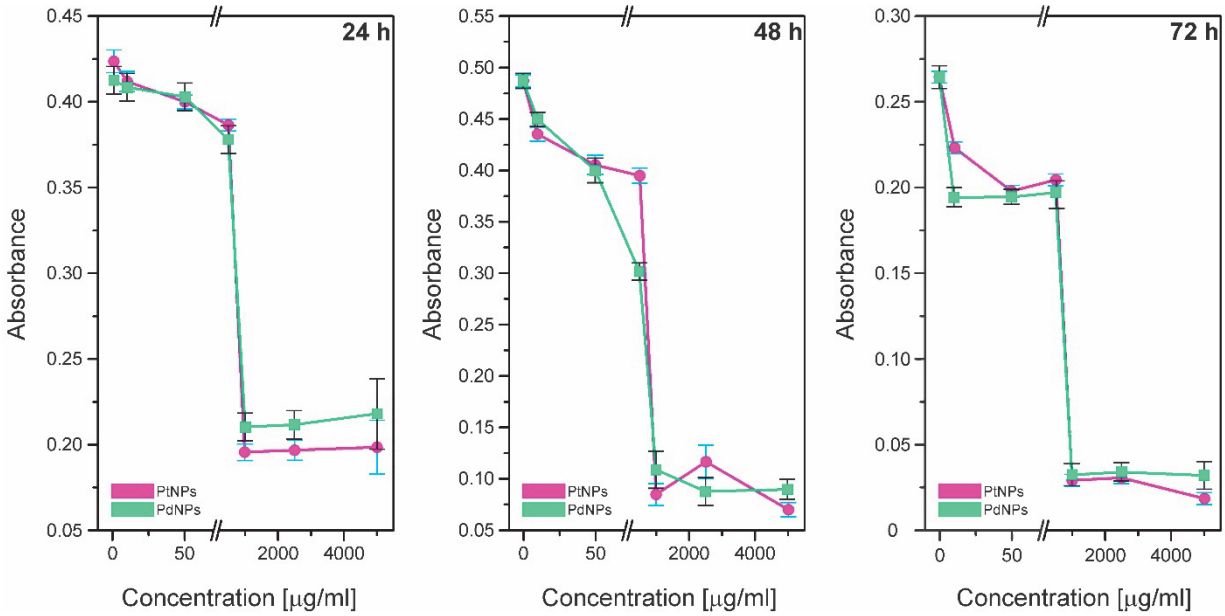

Figure 4 Cytotoxicity of Pd and PtNPs on HEK 293T cells after 24 h, 48 h and 72 h of treatment

4. CONCLUSION

To sum up, we have successfully prepared Pt and PdNPs by cathode sputtering into glycerol. Mean average diameter of Pt and Pd nanoparticles prepared by this technique was 1.8 ± 0.4 and 2.3 ± 0.4 nm, respectively. Such nanoparticles exhibit pretty narrow size-distribution with good potency against agglomeration. We have also demonstrated a significant difference in antibacterial activities of Pt and PdNPs. More specifically, PdNPs exhibited considerable inhibitory potential against both *E. coli* and *S. epidermidis*, which was in contrast to ineffective PtNPs. Our results indicate that palladium has high potential to combat both Gram-positive and Gram-negative bacterial strains. However, we have also shown high cytotoxic activity against HEK 293T cells caused by both Pt and PdNPs. This in effect restricts the possibility of using PdNPs as a potential substitute for commonly used antimicrobial agents to specific areas of use e.g. in combination with its firm immobilization to only *in vitro* applications.

Observed discrepancy in bactericidal action between Pt and Pd nanoparticles should be predominately attributed to different size of individual particles. This aspect seems to play crucial role in the biological activity assessment according to numerous studies. Nevertheless, the exact mechanism of biological action of metal NPs is still unclear, opening possibilities for further, more extensive research.

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