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Proceeding Paper

Influence of Surface Charge on Biological Behaviour of Gold Nanoparticles in Human SH-SY5Y Neuronal Cells †

Vanessa Valdiglesias [1,2,*], Mónica Paz [3], Assia Touzani [1,2], Sandra Baúlde [3], Jesús Mosquera [3], Alejandro Criado [3], Eduardo Pásaro [2,4], Josefina Méndez [1], Blanca Laffon [2,4] and Natalia Fernández-Bertolez [1,2]

[1] Grupo NanoToxGen, Centro Interdisciplinar de Química e Bioloxía—CICA, Departamento de Biología, Facultad de Ciencias, Campus A Zapateira s/n, Universidade da Coruña, 15071 A Coruña, Spain; assia.touzani@udc.es (A.T.); josefina.mendez@udc.es (J.M.); natalia.fernandezb@udc.es (N.F.-B.)
[2] Instituto de Investigación Biomédica de A Coruña (INIBIC), 15071 Oza, A Coruña, Spain; eduardo.pasaro@udc.es (E.P.); blanca.laffon@udc.es (B.L.)
[3] Grupo Nanoself, Centro Interdisciplinar de Química e Bioloxía—CICA, Departamento de Química, Facultad de Ciencias, Campus A Zapateira s/n, Universidade da Coruña, 15071 A Coruña, Spain; monica.andrea.pazi@udc.es (M.P.); sandra.baulde@udc.es (S.B.); j.mosquera1@udc.es (J.M.); a.criado@udc.es (A.C.)
[4] Grupo DICOMOSA, Centro Interdisciplinar de Química e Bioloxía—CICA, Departamento de Psicología, Facultad de Ciencias de la Educación, Campus Elviña s/n, Universidade da Coruña, 15071 A Coruña, Spain
* Correspondence: vanessa.valdiglesias@udc.es
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Abstract: Gold nanoparticles (AuNP) are one of the most remarkable nanomaterials. Due to their small size, these NP can cross the blood-brain barrier making them good candidates for the treatment of diseases related to the central nervous system. The main objective of the present work was to evaluate the influence of surface charge on biological behaviour of AuNP by assessing the cytotoxic—viability and morphological alterations—and genotoxic—double strand breaks—effects induced in neuronal cells exposed to AuNP with different charge: cationic, anionic and neutral. Different toxicological behaviour was obtained depending on the surface charge of the NP.

Keywords: gold nanoparticles; neuronal cells; surface charge; cytotoxicity; genotoxicity

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1. Introduction

Gold nanoparticles (AuNP) are one of the most remarkable nanomaterials. They have aroused great interest in the last years because of their particular properties and their high potential for biomedical applications [1,2]. Due to their small size, these NP can cross the blood-brain barrier, which makes them good candidates for the treatment of diseases related to the central nervous system [3,4]. Despite these potential benefits, the information about the short- and long-term effects of AuNP in organisms and the environment is very scarce, although several adverse effects have been reported (reviewed in [5,6]). Once AuNP enter the body, their interaction with biological systems has been found to be related to their physicochemical properties, which determine their internalization within cells [5]. The main physicochemical properties that affect AuNPs toxicity include size, surface chemistry, and shape [7]. On this basis, the main objective of the present work was to evaluate the influence of surface charge on biological behaviour of AuNP. Thus, cytotoxic and genotoxic effects induced by AuNP with different charge, i.e., cationic, anionic and neutral, were assessed in neuronal SH-SY5Y cells.

2. Materials and Methods

The three types of AuNP used in the present study were newly synthesized following the method reported by Brust et al. [8]. Average hydrodynamic size and zeta potential of NP in neuron culture medium were determined by dynamic light scattering (DLS) and electrophoretic light scattering (ELS), respectively, using a Zetasizer Nano-ZS (model ZEN 3600, Malvern Instruments Ltd.).

Morphological analysis was performed by employing an inverted light microscope (Nikon Instruments Inc.). Phase-contrast photographs of control and AuNP treated cells were obtained. NP effects on viability were evaluated by MTT assay [9] using a SPECTROstar Nano (BMG Labtech) microplate reader, and analysis of H2AX phosphorylation was carried out by flow cytometry [10] in a FACScalibur cytometer (Becton Dickinson). For all these experiments, SH-SY5Y cells were incubated with the three different AuNP at a range of concentrations or the control solutions, for 3 and 24 h.

Differences among groups were statistically analysed by Kruskal-Wallis test, and Mann-Whitney *U*-test for two-by-two comparisons, by employing SPSS for Windows statistical package (version 20.0). The associations between two variables were analysed by Pearson's correlation. Experimental data were expressed as mean ± standard error and a *p*-value of <0.05 was considered significant. All experiments were run at least in triplicate.

3. Results and Discussion

3.1. Nanoparticle Characterization and Cellular Uptake

The AuNP employed in the present study are 2–4 nm spherical NP with positive (cationic), negative (anionic) or neutral surface charge. Results obtained from the analysis of hydrodynamic size and zeta potential of these NP are collected in Table 1. Dispersion of AuNP resulted quite stable and similar for all of them, with almost no variations in the hydrodynamic sizes. The zeta-potential values confirmed the charge of the coating of the NP and supported their stability in suspension.

Table 1. Physical-chemical characterization of AuNP.

	Cationic	Anionic	Neutral
Hydrodynamic diameter (nm) [a] (DLS)	5.46 ± 2.840	4.71 ± 0.900	2.71 ± 0.620
Zeta potential (mV) [a] (ELS)	35.8 ± 1.76	−26.4 ± 1.60	−3.18 ± 1.34

[a] Mean ± standard deviation. DLS: Dynamic Light Scattering, ELS: Electrophoretic Light Scattering.

3.2. Morphological Alterations after AuNP Exposure

No morphological changes in neuronal cells were found after treatment with anionic or cationic AuNP for the selected exposure times. In the case of neutral AuNP, morphological alterations were only detected after 24 h of exposure at the highest concentrations and included rounding of the cells, loss of neurites and slight detaching from the surface. Example photomicrographs are shown in Figure 1.

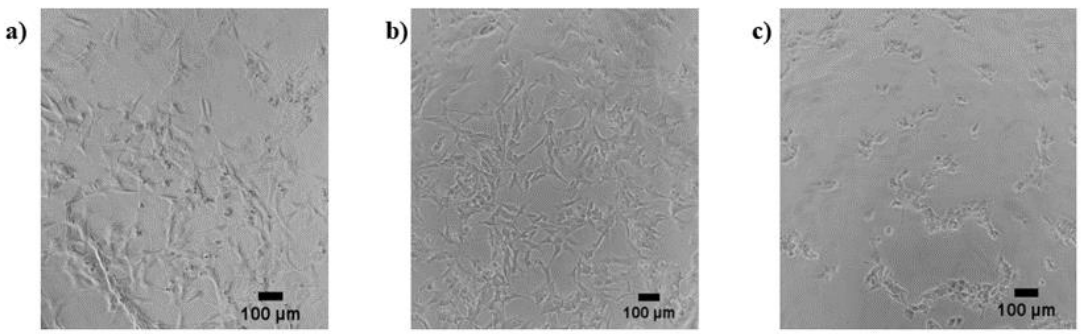

Figure 1. SH-SY5Y neuronal cells without treatment (**a**) and treated with 0.5 μg/mL (**b**), and 50 μg/mL (**c**) of neutral AuNP.

3.3. Viability of Neuronal Cells Exposed to AuNP

The effects of AuNP exposure on viability of neuronal SH-5YSY cells were evaluated by means of MTT assay. Following Costa et al. [11], a modified MTT protocol was employed to avoid any potential interference of the NP. Results from these experiments are shown in Figures 2–4. Although slight but significant decreases in viability were observed for anionic AuNP treatments (Figure 2), this cannot be considered as cytotoxic effects according to ISO 10993-5 [12], since the reductions in cell viability were not higher than 30%.

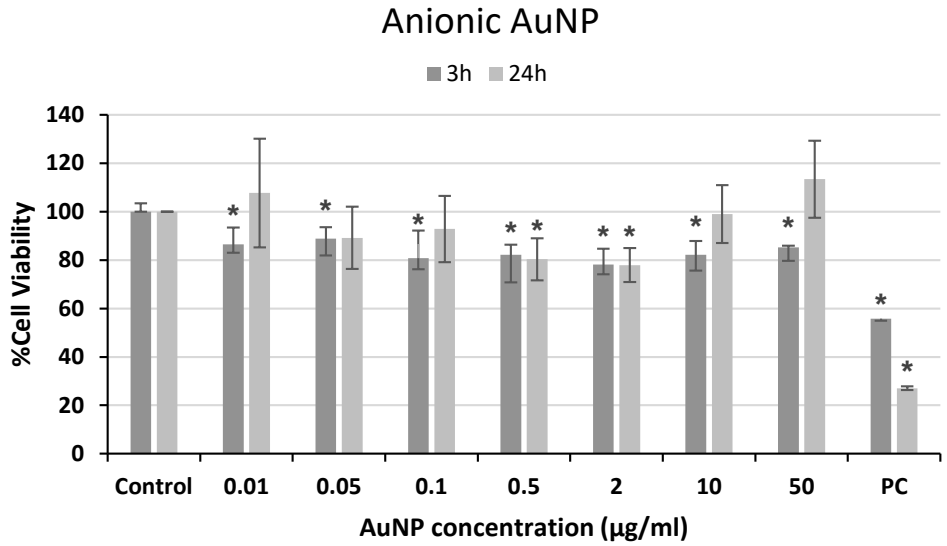

Figure 2. Cell viability of human neuroblastoma SH-SY5Y cell line after exposure to anionic AuNP for 3 and 24 h. PC: positive control (1% Triton 100-X). * $p < 0.05$, significant difference regarding the corresponding negative control.

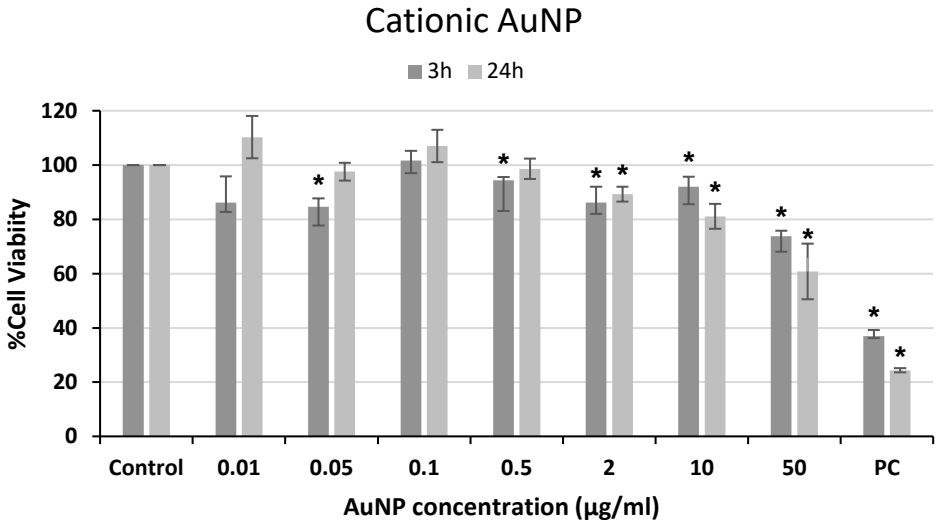

Figure 3. Cell viability of human neuroblastoma SH-SY5Y cell line after exposure to cationic AuNP for 3 and 24 h. PC: positive control (1% Triton 100-X). * $p < 0.05$, significant difference regarding the corresponding negative control.

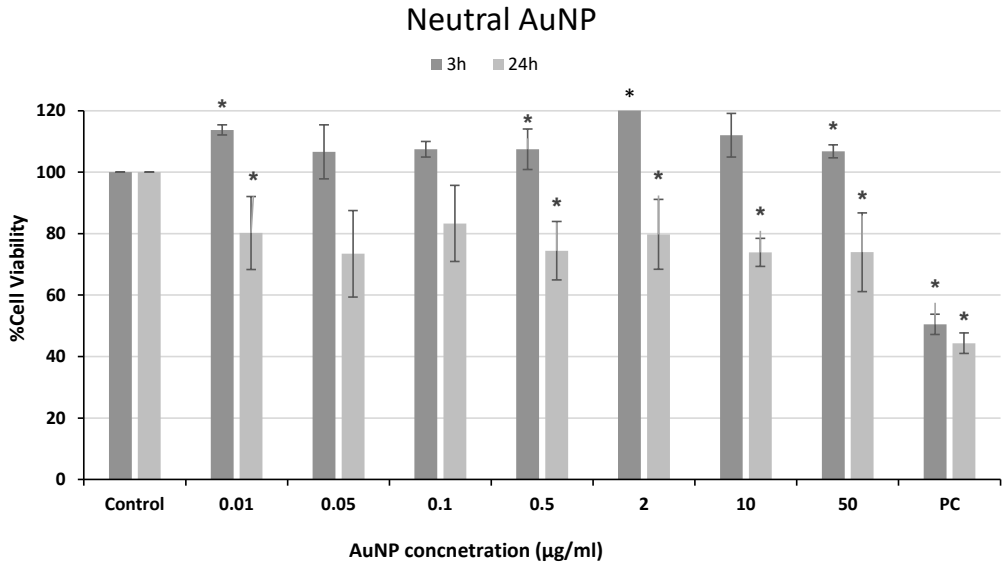

Figure 4. Cell viability of human neuroblastoma SH-SY5Y cell line after exposure to neutral AuNP for 3 and 24 h. PC: positive control (1% Triton 100-X). * $p < 0.05$, significant difference regarding the corresponding negative control.

A decrease in cell viability was observed after cationic AuNP treatment at the highest doses in both exposure times (Figure 3), reaching values of 70% and 60% of viability at the highest concentration employed after 3 or 24 h, respectively. However, a statistically significant dose-response relationship was only found for 2 4 h treatment ($r = -0.795$; $p < 0.01$).

For neutral AuNP exposure, significant decreases in cellular viability regarding the negative control were found only after 24 h treatment, with values around 70–80% at all concentrations tested (Figure 4).

3.4. Genotoxic Effects of AuNP

Results obtained from the analysis of H2AX phosphorylation of neuronal SH-SY5Y cells exposed to anionic, cationic or neutral AuNP are shown in Figures 5–7. Slight increases were observed in the percentage of cells with γH2AX at all the concentrations tested for anionic AuNP, although values registered always maintained below 10% (Figure 5).

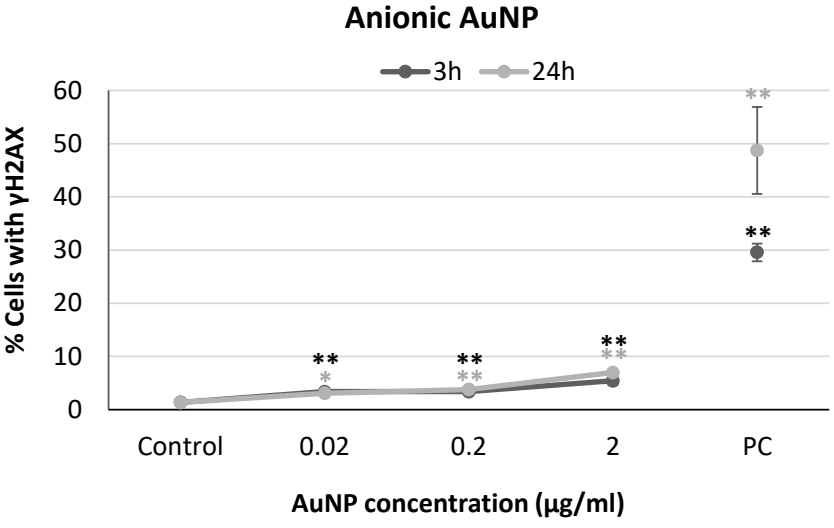

Figure 5. Results from yH2AX analysis in SH-SY5Y cells exposed to anionic AuNP for 3 and 24 h. PC: positive control (1 μg/mL BLM). * $p < 0.05$, significant difference regarding the corresponding negative control.

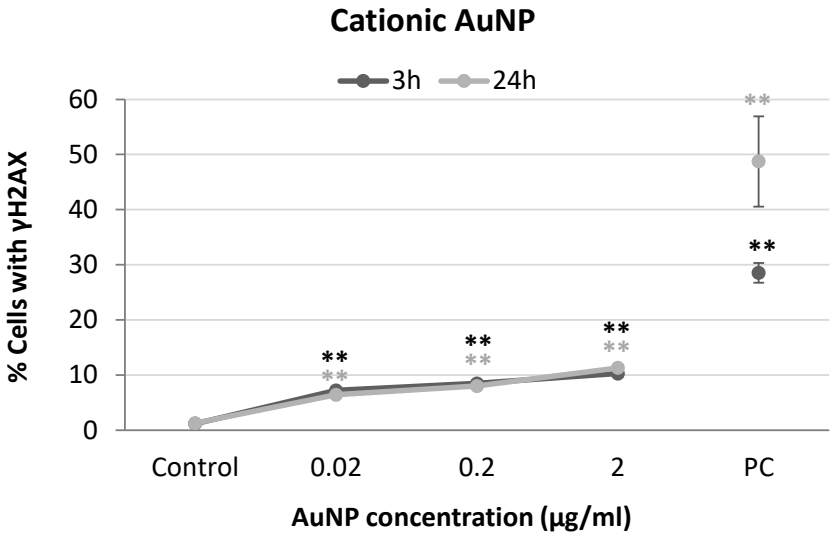

Figure 6. Results from yH2AX analysis in SH-SY5Y cells exposed to cationic AuNP for 3 and 24 h. PC: positive control (1 μg/mL BLM). * $p < 0.05$, significant difference regarding the corresponding negative control.

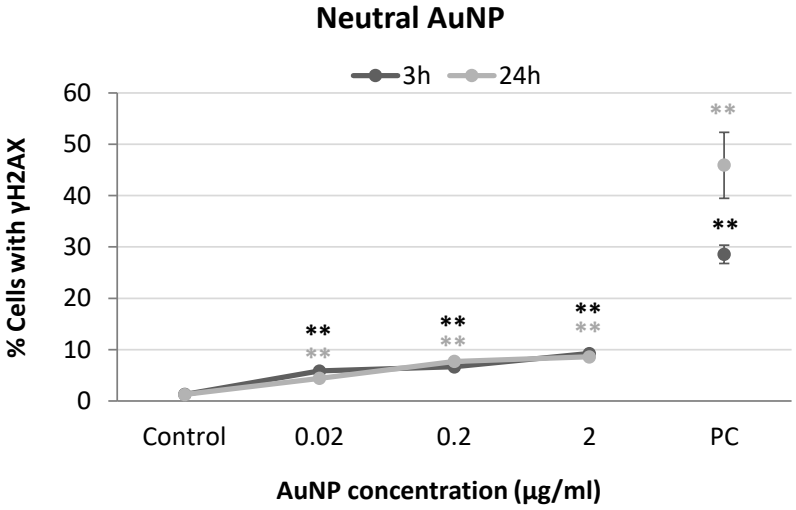

Figure 7. Results from yH2AX analysis in SH-SY5Y cells exposed to neutral AuNP for 3 and 24 h. PC: positive control (1 μg/mL BLM). * $p < 0.05$, significant difference regarding the corresponding negative control.

Dose-dependent increases in the percentage of cells with γH2AX were observed in neuronal cells treated with cationic AuNP after both exposure times (3 h: r = 0.692; $p < 0.01$; 24 h: r = 0.900; $p < 0.01$) although more notable for 24 h (Figure 6).

Finally, significant increases in γH2AX levels were obtained for all conditions tested when SH-SY5Y were exposed to neutral AuNP (Figure 7). Concentration-dependent relationships were observed also in this case for both exposure times (3 h: r = 0.824; $p < 0.01$; 24 h: r = 0.884; $p < 0.01$).

4. Conclusions

Results obtained from this work highlighted the relevance of surface charge on the AuNP toxicological behaviour. In particular, anionic and neutral AuNP did not cause cytotoxic effects, while cationic nanoparticles showed cytotoxicity at the longest exposure time. Furthermore, cationic and neutral AuNP showed just a moderate genotoxic potential at 24 h treatments, while those with a negative charge did not induce a remarkable amount of double-strand breaks in DNA at any condition tested.

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