Effect of size on the toxicity of gold nanoparticles

Noelia Alvarez [(1,2)], Ane Ayerdi [(1,2)], Leire Goikoetxea [(1,2)], Jaione Lorenzo [(1,2)], Ruben Fernandez [(1,2)], Nerea García [(1,2)], Ainhoa Egizabal[(1,2)]

(1) TECNALIA. Mikeletegi Pasealekua 2, E- 20009 Donostia (Spain)
(2) Ciber - BBN (Spain)
Ainhoa.egizabal@tecnalia.com

Abstract

The last decade has seen an important growth in the production of nanoscale materials as a result of their attractiveness for a large range of applications in biomedicine[1], biosensing[2, 3], microelectronics[4], material engineering[5], energy production[6,7] and environment remediation[8,9]. Gold nanoparticles (AuNPs) have attracted particular scientific and technological interest due to their unique optical properties, chemical stability, easy synthesis and functionalization, all of which make AuNPs interesting candidates to use in biomedicine. However, knowledge about the health impact of gold nanoparticles is essential before these nanomaterials can be used in real clinical settings[10]. Many scientific reports have been published addressing this issue, with the goal of understanding the interactions between nanoparticles and cells as function of their size, shape, and surface chemistry[3, 11]. Although AuNPs are considered inert particles and regarded as biocompatible, there are contradictory results concerning their toxicity[12]. The goal of this work is to provide additional data on the toxic potential exerted by AuNPs of different sizes. We investigated the effects of gold nanoparticles of 5 and 20 nm on NIH/3T3 mouse fibroblast, the hemocompatibility and systemic toxicity. Finally, the biodistribution and bioaccumulation of the AuNPs *in vivo* into the lung, liver, spleen and kidney were examined.

The cytotoxicity in NIH/3T3 mouse fibroblast cells (ATCC, CRL-1658) was determinated using MTT assay. Freshly prepared 5 and 20 nm AuNPs were dispersed in cell culture medium, diluted at concentrations from 425 µg/ml to 6.64 µg/ml and were added to cells. After 4 h of treatment, MTT assay was carried out to obtain cell viability (%). The Hemocompatibility was evaluated based on ASTM E2524-08 (Standard Test Method for Analysis of Hemolytic Properties of Nanoparticles) through a hemolysis test and an evaluation of blood coagulation. A Test for Systemic Toxicity was performance under ISO 10993-11 Standard. Fifteen Winstar Hannover female ratswere randomly divided into three groups: one control group and two experimental groups exposed to the selected nanoparticles.. Rats received intraperitoneal (i.p.) injections of 100 µL of AuNP during 6 days. Control group was treated with vehicle solution (1.2 mM sodium citrate). The body weight of the animals and their behavior were carefully recorded daily during the course of the experiment. One day after the last injection (day 7), rats were sacrificed, and the lung, liver, spleen and kidney were collected immediately. A part of the organs was stripped for histological evaluation. The remaining samples were stored at -80ºC for the quantification of gold content in each tissue though ICP-AES analysis and for the evaluation of the RNA integrity of the organs.

The cell viability of 3T3 fibroblast exposed to 5 and 20nm AuNPs was higher than 80% in all of the assayed concentrations Nevertheless, the viability of 3T3 fibroblasts exposed to 5 nm AuNPs was lower. The hemocompatibility assays showed that 5 nm AuNPs had an haemolytic effect, in contrast with the 20 nm AuNPs samples. The PT and aPTT values for 5 nm and 20 nm samples were greater than reference values, indicating that samples affected the blood clotting. After testing if AuNPs treatment produces sub-acute toxicity in rats during the course of the study, we observed no mortality and no weight or any behavioral changes in the rats receiving 5 and 20 nm AuNPs.The histological evaluation of the liver tissue did not show any damage in rats exposed to 5 nm and 20 nm AuNPs. Nevertheless, ICP-AES results showed a significant increase in the amount of gold in liver and spleen after repeated injection of AuNPs in comparison with control groups.. Moreover, the bioaccumulation of gold in the organs of rats treated with 5 nm AuNPs was greater than in rats treated with 20 nm AuNPs. Finally, the values of RNA integrity number (RIN) obtained from the lung and the kidney of control rats and rats treated with 20 nm AuNPs were greater than 5, suggesting that RNA was not degraded. On the contrary, the electropherograms and RNA integrity numbers (RIN) obtained from the lung and the kidney of rats treated with 5 nm AuNPs, showed a degradation of the RNA.

References

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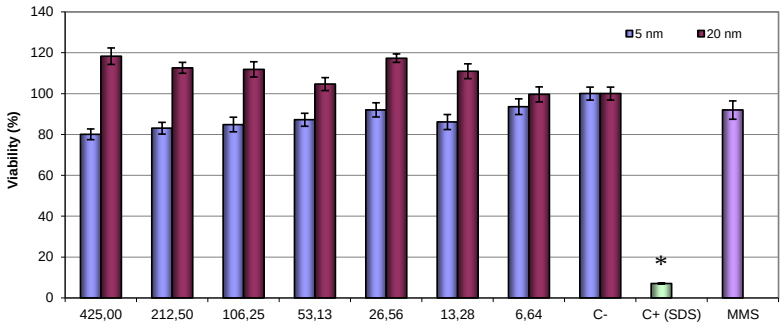
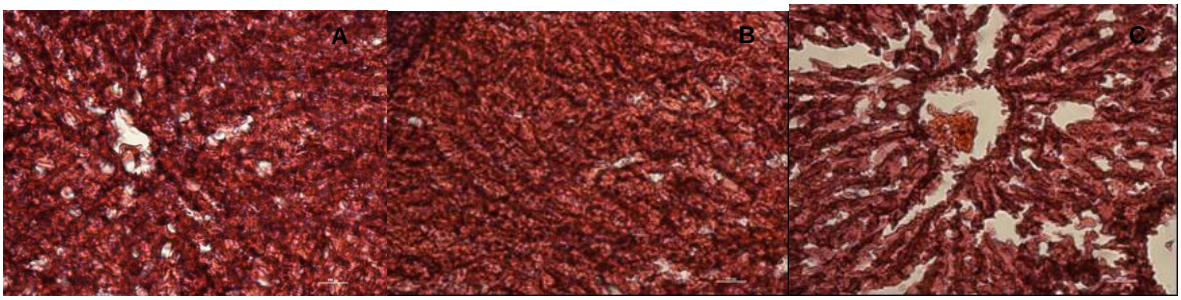
Figures



*Viability of 3T3 fibroblasts after 4 hours in contact with AuNP of 5 nm and 20 nm (*p<0.05)*

Sample	Haemolytic index (%)
Negative control	2,6 ± 1,1
Positive control	104,3 ± 3,1
AuNP-5nm	10,3 ± 1,9
AuNP-20nm	1,9 ± 1,4

Haemolytic index (%) of the AuNP samples and controls



Histological evaluation of Liver stained with haematoxilyn-eosin obtained from A) Control animal, B) Animal treated with AuNP of 5nm and C) Animal treated with AuNP of 20nm (X 40)

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