EXPLORING THE IMPACT OF METALLIC NANOPARTICLES ON BIOLOGICAL SYSTEMS BY ICP-MS AND COMPLEMENTARY TECHNIQUES

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Introduction.

The steadily increasing use of nanomaterials in our daily life requires the assessment of their potential impacts on any biological system. Significant data on their biological pathways, their degradation, and their potential toxicity on humans and other organisms are needed in order to guarantee their safe use. Among them, gold nanoparticles (AuNPs) show various features that are very interesting for applications in cosmetics, catalysis, and in biomedicine.

In order to explore the possibilities and limitations of any nanomaterial useful and harmless for commercial products, several tests are required [1]. In this context, our interest has been to explore the pathway, accumulation, and physiological impact of AuNPs in biological systems like cells and model organisms. With the use of citrate-stabilised AuNPs of different sizes, we have concentrated on three aspects being important for the potential use of such nanomaterials in wider applications. Once in contact with a biological specimen, AuNPs have been explored for i) their potential degradation accompanied by the release of low-molecular gold species, ii) their physiological and toxicological impact by various *in vitro* and *in vivo* tests, and iii) the formation and composition of the protein corona.

Degradation of AuNPs after their incorporation in biological systems.

Nanomaterials can undergo various transformations upon any changes in their environment. Such processes might include agglomeration, aggregation and/or degradation among others. The release of low-molecular metal species usually accompanies the degradation of metallic nanoparticles like AuNPs. Their occurrence, e.g. in biological system, strongly influence the uptake, the localisation, and the toxicological behaviour in the host. Therefore, their detection is of utmost importance. In order to discriminate between these different metallic forms (NPs or low-molecular species) analytical techniques are needed for a detailed insight. Inductively coupled plasma-mass spectrometry (ICP-MS), well known as a very sensitive and selective technique for the determination of a huge number of elements, offers here various possibilities. Similar to elemental speciation analysis, hyphenated techniques like high performance liquid chromatography (HPLC) [2] or capillary electrophoresis (CE) [3] coupled to ICP-MS have emerged to tackle this analytical challenge. Moreover, the early development of single particle-ICP-MS (sp-ICP-MS) has paved the way for nowadays emerging application in nanoparticle characterisation with simultaneous determination of released metal species [4].

Based on a method using an enzymatic digestion with proteinase K in combination with HPLC-ICP-MS various cell lines and tissues from Wistar rats after exposure to differently sized AuNPs were analysed for their degradation. The differences between *in vitro* and *in vivo* studies were clearly notable. Whereas the incubated AuNPs in the cell studies remained intact [5], a pronounced degradation, thus the formation of low-molecular gold species was observed in rat tissues like liver or spleen samples [5-7]. A typical chromatogram obtained from an extract of a spleen tissue reveals this finding (Figure 1). The signal at 4.8 min represented the AuNPs initially injected (about 10 nm diameter) still observable in the tissue. Transmission electron microscopy (TEM) as complementary technique confirmed this finding [6]. However, the second eluting fraction at 5.7 min revealed the presence of gold species as degradation products from the AuNPs. Until now, it is not clear whether this degradation occurred during the transport of the AuNPs to the different organs or during their accumulation. Further studies are needed here to get deeper insight into these processes; nevertheless these observations might have an impact on the physiological impact of AuNPs applied in biomedical applications.

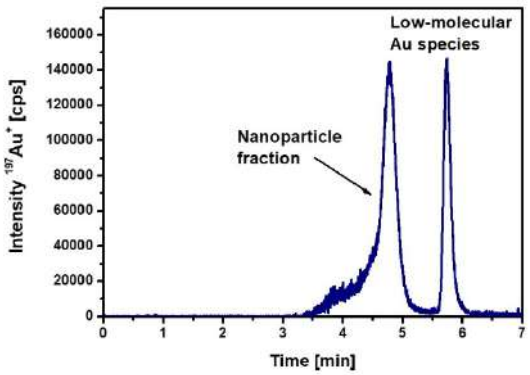

Figure 1: *HPLC-ICPMS chromatograms of a spleen sample digested with proteinase K overnight from a rat injected intraperitoneally with 10 nm Au NPs. (Reproduced from [6], with permission from the Royal Society of Chemistry)*

Physiological and toxicological impact of AuNPs.

Despite the potential benefits of AuNPs in areas of biomedical applications, there has been an increased interest in studying their possible deleterious effects in biological systems. Different parameters such as particle diameter, surface coating, shape, dose or route of administration have shown to be essential for the distribution, accumulation, metabolism, elimination, and therefore for any physiological and toxicological effects of AuNPs.

Within these studies, HT-29 and HepG2 cell lines and Wistar rats were exposed to 10, 30 or 60 nm gold nanoparticles to determine their tissue distribution, subcellular location and deleterious effects. Cell viability, ROS production and DNA damage were evaluated *in vitro*. Lipid peroxidation and protein carbonylation were determined in liver. These physiological tests were supported by ICP-MS measurements. Gold was present in various organs and less pronounced in faeces and urine of the investigated Wistar rats, whereas highest concentrations were observed in spleen depending on the administrated particle size [5]. In cell studies, nanoparticles being able to enter the cell nucleus and thus producing elevated DNA damage was only observed for the smallest administrated particles of 10 nm. Figure 2 demonstrates a TEM image that shows a nanoparticle inside the cell nucleus.

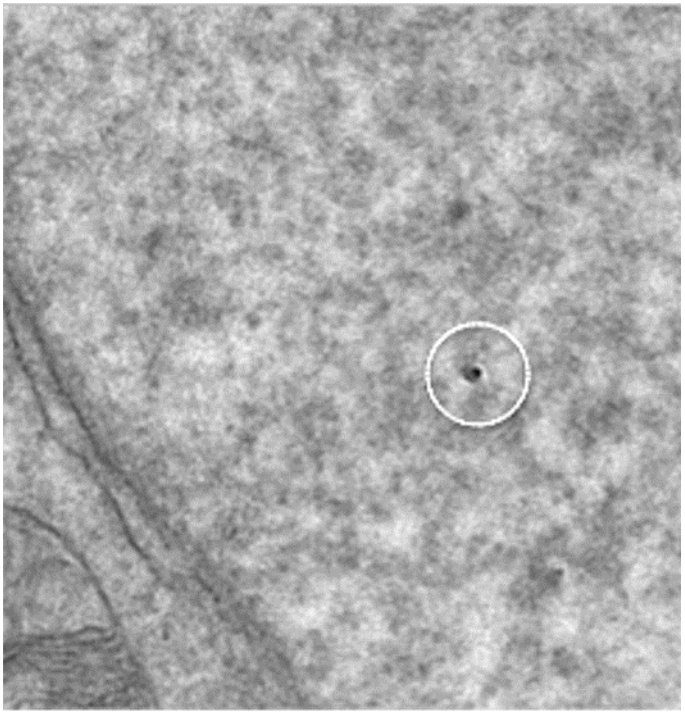

Figure 2: *TEM image from colon cells treated with 10 nm AuNPs. (Reproduced from [5], with permission from Elsevier)*

The overall results showed that the administrated AuNPs increased the production of reactive oxygen species (ROS) with an enhanced damage in proteins and lipids. However, this increase was not sufficient to trigger any inflammatory response and therefore any tissue damage.

The formation and composition of the protein corona.

For better understanding of the physiological impact of nanoparticles in biological systems, it is also important to take into account another aspect. Once a nanoparticle enters any organism, it is well known that various biomolecules cover its surface [8]. This so-called “protein corona” is believed to determine the cellular response to nanomaterials. A huge number of studies deal with the determination of the composition of the protein corona. For instance, cell cytosols or other liquid body fluids were incubated with the nanomaterials of interest to study the formation, the stability, and the qualitative and quantitative composition of the protein corona [8, 9]. Only few studies exist to reveal the real situation in systems like living cells. The main challenge here is the isolation of the intact

nanoparticle-protein corona bioconjugate from an intact biological system. In this context, we proposed a sample preparation procedure for eukaryotic cells that included a soft lysis followed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of the proteins with subsequent in-gel digestion and identification of the generated peptides by liquid chromatography-electrospray ionisation-tandem mass spectrometry (LC-ESI-MS/MS) [10]. This procedure was adapted, among others, to two human cell lines (HCT-116 and A549) [11].

In total, 297 (HCT-116) and 52 (A549) proteins were identified in the hard protein corona, of which a few originated from the cell medium (bovine proteins). The composition of the protein corona reflected proteins that participated in essential events in the cellular processing of the gold nanoparticles, in particular during their interaction with the outer surface of the cell and their clathrin-mediated endocytosis. The protein composition also indicated the role of the hard corona in induced cellular response mechanisms [11]. A brief summary of observed proteins is given in Figure 3.

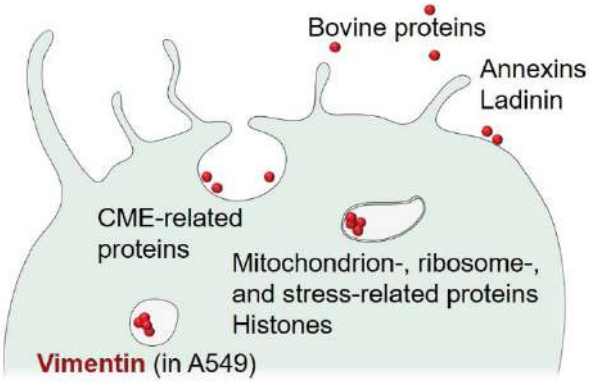

Figure 3: *Summary of the proteins involved in the processing of gold nanoparticles by cells (adapted from [11]).*

These findings were also supported by the fact that in another cell line, J774, protein fragments formed part of the hard protein corona. Our investigations suggested that these fragments were formed after internalization of the nanoparticles by proteolysis of intact proteins [12]. This might indicate that even if such nanoparticles did not trigger any toxicity to the cells, the cells actively recognised them as unnatural “intruder”.

Conclusions.

The aim of this work was to elucidate the pathway and internalization of citrate-stabilised AuNPs. The use of different complementary analytical techniques like ICP-MS, TEM, and ESI-MS enabled us to get some important insights into these processes. The *in vivo* degradation of these nanoparticles and the composition of the protein corona, here at least proven in *in vitro* studies, can be considered as important aspects in future applications of AuNPs.

Acknowledgements.

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References.

[1] A. Sani et al. (2021) Biochem. Biophys. Rep., Art. No. 100991.

[2] A. Helfrich et al. (2006) J. Anal. At. Spectrom. 21: 431-434.

[3] B. Franze, C. Engelhard (2014) Anal. Chem. 86: 5713-5720.

[4] C. Degueldre, P.-Y. Favarger (2003) Colloids Surf. A 217: 137-142.

[5] C. Lopez-Chaves et al. (2018) Nanomed. Nanotechnol. Biol. Med. 14: 1-12.

[6] J. Soto-Alvaredo et al. (2017) J. Anal. At. Spectrom. 32, 193-199.

[7] R. Álvarez-Fernández García et al. (2019) J. Trace Elem. Med. Biol. 55: 1-5.

[8] T. Cedervall et al. (2007) Proc. Natl. Acad. Sci. U.S.A. 104: 2050-2055.

[9] D. Walczyk et al. (2010) J. Am. Chem. Soc. 132: 5761-5768.

[10] G.P. Szekeres et al. (2020) J. Proteom. 212: 103582.

[11] G.P. Szekeres et al. (2020) Nanoscale 12: 17450-17461.

[12] G.P. Szekeres et al. (2020) Anal. Chem. 92: 8553-8560.