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The unique properties of gold nanoparticles (AuNPs), such as their multifunctionality potential (ranging from clinical diagnostics to therapeutics), make them highly attractive [1].

The current work aimed at i) synthesizing and characterizing AuNPs of different shape (stars vs spheres), size and surface characteristics, and further ii) assessing the in vivo distribution of the novel synthesized agents, and iii) assessing the influence of size, shape and coating agent on the in vitro toxicological effects.

By using different methodologies based upon seed-mediated growth synthesis, spherical and star-shaped AuNPs were synthesized and coated with 11-mercaptoundecanoic acid (MUA) or with sodium citrate. Transmission electron microscopy (TEM), dynamic light scattering (DLS), and UV-Vis spectrophotometry were employed for the characterization of the AuNPs. The gold concentration of the samples was obtained by graphite furnace atomic absorption spectrometry (GFAAS) and the concentration of nanospheres was determined using the UV-Vis spectrum, based on the mathematical equation of Haiss et al. [2]. The effect of the shape on the AuNPs biodistribution was evaluated on Wistar rats. A dose of 0.6 mg Au/ kg of MUA-coated gold nanostars (54 nm of diameter) or of citrate-coated gold nanospheres (58 nm of diameter) was given per os and the quantification of gold was determined on different organs and biological fluids was assessed 24h later. Also, the toxicity of the AuNPs was evaluated in vitro using HepaRG cells (non-differentiated and differentiated for 15 days with 2% dimethyl sulfoxide), Caco-2 cells and primary rat hepatocytes. The performance of two distinct viability assays, namely (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium (MTT) reduction and neutral red (NR) incorporation assays, was assessed after 4 and 24 hour incubations. In the case of Caco-2 cells, one additional assay, i.e. the lactate dehydrogenase (LDH) release assay, was also performed. Six concentrations of each treatment (1 µM, 5 µM, 10 µM, 20 µM, 40 µM, and 60 µM) were tested. Solvent (2.2 mM sodium citrate and 33 µM MUA), negative (cell culture media) and positive (1% triton-X100) controls were also included in each experiment.

Three batches of MUA-capped gold nanostars ranging from 54 nm to 72 nm of diameter and three batches of citrate-capped gold nanospheres (diameter from 15 nm to 67 nm) were produced. For the sake of comparison MUA-capped gold nanospheres of 15 nm were also synthesized. Preliminary in vivo data demonstrated that for both types of gold nanoparticles, low levels of AuNPs were detected in the biological samples (in the majority of organs analyzed the data was below the limit of the quantification of the method). In what concerns the toxicity of the synthesized AuNPs, our results indicated detrimental effects on all the tested cellular models, at the highest concentrations. The toxicity profiles of the tested AuNPs were not the same for all cell lines but in all cases a concentration-dependent relationship was established. Caco-2 enterocytes proved to be the most resistant model, while the rat primary and HepaRG hepatocytes were the most sensitive, suggesting that metabolism is not involved in the observed toxicity. Regarding the shape, nanospheres showed higher toxicity, when compared with the stars. The star-shaped NPs with higher diameter displayed greater injuriousness than smaller NPs in HepaRG non-differentiated and in primary rat hepatocytes. In what concerns the coating agent, neither MUA or sodium citrate seem to affect the toxicological profile of gold NPs.

In respect to their toxicity, these preliminary results suggest that our novel gold nanomaterials have high potential to be considered promising candidates for industry, but further investigations are required.
particularly aiming at elucidating the oral biodistribution profile, when different doses of the AuNPs are administered.

References


Figures

![Figure 1](image1.png)  ![Figure 2](image2.png)

Figure 1. Synthesis of gold nanoparticles.  Figure 2. Synthesis of gold nanoparticles.

![Figure 3](image3.png)  ![Figure 4](image4.png)

Figure 3. In vitro assessment of cytotoxicity.  Figure 4. Experimental results of toxicological and biodistribution assays.