

Comparative Studies on Cytotoxicity of Gold Nanoparticles and Protein-Stabilized Fluorescence Gold Nanoclusters

**Patrycja Gnacek¹, Anna Barbasz², Ditta Ungor³, Agnieszka Czyżowska², Edit Csapó^{3,4},
Julia Maciejewska-Prończuk¹, Paulina Żeliszewska¹, Magdalena Oćwieja¹**

¹Jerzy Haber Institute of Catalysis and Surface Chemistry Polish Academy of Sciences, Niezapominajek 8,
PL-30239 Krakow, patrycja.gnacek@ikifp.edu.pl, julia.maciejewska-pronczuk@ikifp.edu.pl,
paulina.zeliszewska@ikifp.edu.pl, magdalena.ocwieja@ikifp.edu.pl

² Institute of Biology, Pedagogical University, Podchorążych 2, PL-30084 Krakow, Poland, anna.barbasz@up.edu.pl

³MTA-SZTE Lendület „Momentum” Noble Metal Nanostructures Research Group, University of Szeged,
H-6720 Rerrich B. sqr. 1, Szeged, Hungary ungord@chem.u-szeged.hu, tidecs2000@yahoo.co.uk

⁴Interdisciplinary Excellence Center, Department of Physical Chemistry and Materials Science,
University of Szeged, H-6720 Rerrich B. sqr. 1, Szeged, Hungary

Gold nanoparticles (AuNPs) and nanoclusters (AuNCs) are interesting materials due to their potential biocompatibility, size- and structure-tunable optical properties. AuNCs and AuNPs could penetrate across the blood-brain barrier and be excreted mainly through the kidney [1]. It was established that AuNCs and AuNPs can inhibit the processes of protein fibrillization, which leads to the formation of numerous senile plaques responsible for neurodegenerative diseases [1]. For these reasons gold nanostructures seem to be ideal candidates for biological and medical purposes. Nevertheless, their potential application in living systems should be preceded by intensive research on effects appearing on the cellular level during the short- and long-term exposure on them [1,2]. Therefore, the aim of these studies was the evaluation of cytotoxicity of AuNPs and AuNCs towards two types of cells of the immune system, namely lymphocytes B (COLO-720L) and lymphocytes T (HUT-78).

The AuNPs were prepared using trisodium citrate (TC) or a mixture of sodium borohydride (SH) and cysteamine hydrochloride (CH). The AuNPs were characterized by quasi-spherical shape and average size within the range 9-12 nm. CHSBAuNPs were positively charged whereas TCAuNPs were negatively charged. The AuNCs were prepared based on the one-pot template-assisted methods using lysozyme (LYZ), human (HSA) and bovine (BSA) serum albumins, and gamma globulin (γ G) as stabilizing agents. Each type of AuNCs exhibited intense red emission ($\lambda_{em} \sim 650$ nm) and size of metal core equal to ca. 1.4 nm. The AuNCs were negatively charged at pH 7.4.

During the exposure of lymphocytes on the AuNPs and AuNCs, the changes in mitochondrial activity, membrane integrity, secretion of inflammatory and the apoptosis mediators of cells were evaluated. It was found that the AuNPs were more toxic for the lymphocytes than the AuNCs but significant differences between positively and negatively charged AuNPs were not noticed. It was established that γ G-AuNCs induced the highest disorders in mitochondrial activity, but the influence of other AuNCs on the cell viability was minor. The secretion of malonic dialdehyde (MDA) was enhanced by LYZ- and γ G-AuNCs. Apart from LYZ-AuNCs, the clusters did not exhibit strong proinflammatory and apoptotic properties. The enhanced secretion of tumor necrosis factor (TNF- α) by lymphocytes B, in comparison to control, was independent of the cluster type. It was established that HSA- and BSA-AuNCs were the least toxic for the lymphocytes. The highest toxicity was determined for LYZ-AuNCs. Because the dependencies between the activity of the proteins and toxicity of Au NCs obtained with their use were not recognized, it was hypothesized that the biological properties of proteins can evolve during the cluster synthesis. Therefore, obtained fluorescent protein-stabilized AuNCs possess completely new biological activities, which should be predicted considering effects coming from pure proteins and gold.

References

- [1] D. Ungor, A. Barbasz, A. Czyżowska et al. in *Colloids and Surfaces B: Biointerfaces*, 2021, 200, 111593.
- [2] A. Czyżowska, A. Barbasz, L. Szyk-Warszyńska et al. in *Colloids and Surfaces A*, 2021, 620, 126569.