Biocompatibility of γ-Fe_2O_3 nanoparticles with blood cells

Paryzhak S.Ya.[1], Dumych T.I.[1], Zasońska B.A.[2], Svoboda J.[2], Klyuchivska O.Yu.[3], Skorokhyd N.R.[3], Kozak Yu.S.[3], Horák D.[2] & Stoika R.S.[1,3]

[1] Danylo Halytsky Lviv National Medical University; [2] Institute of Macromolecular Chemistry, Czech Academy of Sciences; [3] Institute of Cell Biology, National Academy of Sciences of Ukraine

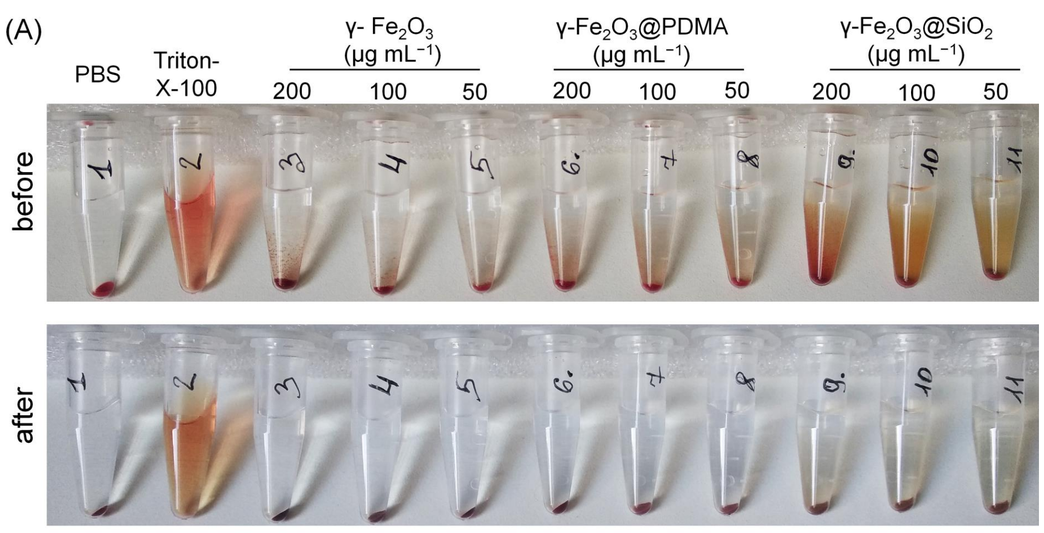
E-mail: sola.paryzhak@gmail.com

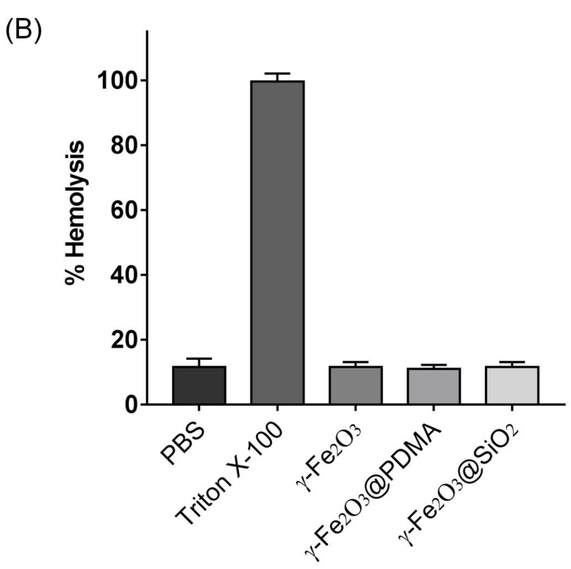
Introduction: The importance of nanotechnologies for industry, power engineering, IT, medicine and other fields is constantly growing. Because of their small size, nanoparticles (NPs) penetrate into systemic circulation, quickly spread throughout the body and overcome biological barriers [1]. Thus, blood cells are the first contacts for the NPs entering the organism [2].

Our research **was aimed** at interaction between γ-Fe_2O_3 NPs (~9 nm) and their poly(N,N-dimethylacrylamide) and SiO_2 coated derivatives with blood cells.

Methods: Erythrocyte damage was determined by means of spectrophotometric method by evaluation of released hemoglobin (Hb) content. Human granulocytes were isolated from the freshly obtained heparinized venous blood of normal healthy donors (approved by the Bio-Ethics committee at the Institute of Cell Biology, NAS of Ukraine (protocol No 2/07102020) using ficoll-triombrast medium with gradient density ρ = 1.08 g/cm^3. For *in vivo* toxicity study, mice were injected intravenously with 5 mg/kg of various NPs. The body weight of mice was weighed twice per week for 2 weeks. All the works including housing and care, method of euthanasia were conducted in accordance with the established experimental protocols and requirements of Ethics committee of Institute of Cell Biology NAS of Ukraine, protocol № 3/07102020.

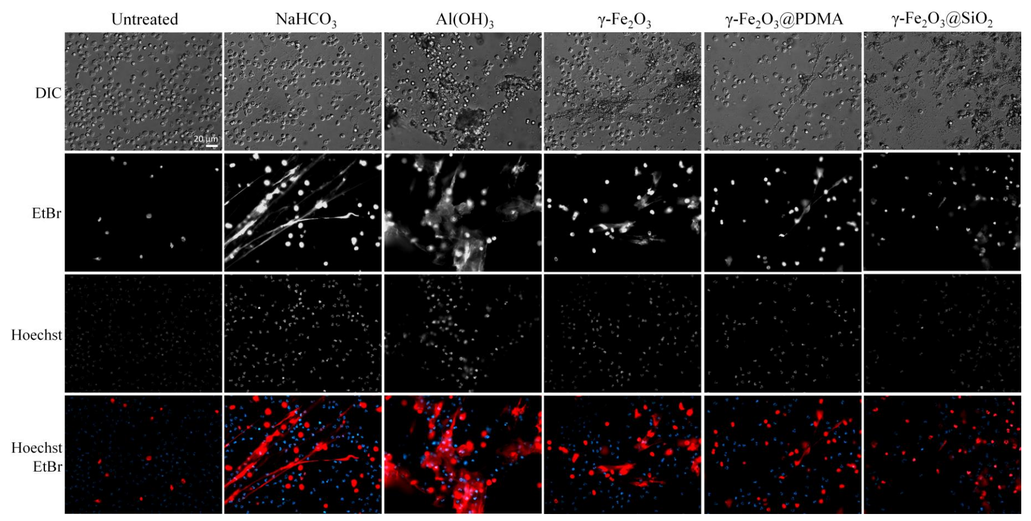
Results: To assess the impact of γ-Fe_2O_3 NPs on red blood cells (RBC), hemolysis test was carried out with the negative control of the PBS buffer and positive control of 1% Triton X-100. Although, different NPs together with RBCs slightly, adhered to the walls of the microcentrifuge tubes, this adhesion did not lead to damaging of RBC with Hb release.





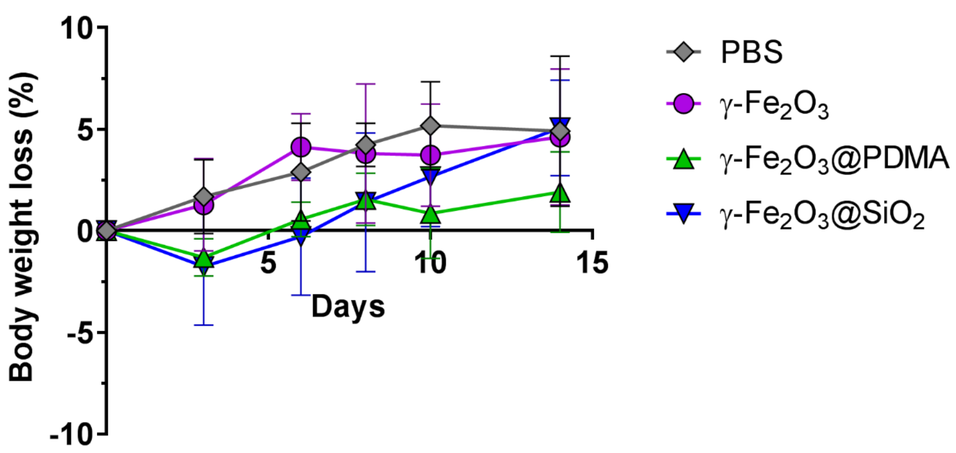
The results of the spectrophotometric measurement of the released Hb after exposure to the tested samples were at the level of the negative control. However, it was significantly different from the results in the positive control.

(A) Visual examination of the tubes containing diluted total blood under exposure to γ-Fe_2O_3, γ-Fe_2O_3@PDMA and γ-Fe_2O_3@SiO_2 for 4 h before or after centrifugation.
(B) Percentage of hemolysis caused by tested NPs.



The investigated NPs caused much lower activation of the neutrophils compared to positive controls (48 mM $NaHCO_3$ and 30 mM $Al(OH)_3$).

DIC imaging of the cells is shown in the upper row. Extracellular DNA was stained with the ethidium bromide (EtBr, red fluorescence). Cell nuclei were stained with Hoechst 33342 (blue fluorescence).



Intravenous administration of NPs to laboratory animals did not manifest any toxic effect and no signs of distress such as behavioral changes, were observed.

In vivo toxicity of different NPs in BALB/c mice. Change in the body weight of mice injected with various NPs at 5 mg/kg dose, compared with PBS control.

Conclusion: In this study, we found out that γ-Fe_2O_3 NPs with the poly(*N,N*-dimethylacrylamide) or silica shells demonstrated high biocompatibility with white and red blood cells *in vitro*, as well as non-toxicity towards cells of the cardiovascular systems in *in vivo* experiments. Well-controlled particle size and morphology are important for the biomedical usage of the tested NPs. In contrast with other nanocomposites described in the literature, the tested NPs are quite small and they can be functionalized with different biomolecules. It makes them promising agents for further applied research.

References:

1. de la Harpe K.M., Kondiah P.D., Choonara Ya.E., Marimuthu T., du Toit L.C., and Pillay V. The Hemocompatibility of Nanoparticles: A Review of Cell-Nanoparticle Interactions and Hemostasis // Cells.-2019.-8.-1209.
2. Matus M.F., Vilos C., Cisterna B.A., Fuentes E., Palomo I. Nanotechnology and primary hemostasis: Differential effects of nanoparticles on platelet responses // Vascul Pharmacol.-2018.-101.-P. 1-8.

Acknowledgements: This work was supported by the Czech Science Foundation (No. 20-02177J).