«Synthesis and application of nanoparticles of complex iron oxides in the study of cellular structures using transmission electron microscopy»

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Abstract. The study of the distribution and interaction of biomolecules (proteins, DNA, RNA, etc.) among themselves in the cell for various biochemical applications is an urgent task that has not been fully solved to date. Traditional methods of biomolecule localization are fluorescence microscopy methods, the resolution of which is limited by the diffraction limit and does not exceed 200-300 nm. The development of super-resolution confocal microscopy methods has led to the possibility of obtaining images of the object with improved resolution up to 20-50 nm, but even this resolution does not allow visualizing the majority of biomolecules whose size is from 1 to 20 nm. It became possible to obtain images with a resolution lower than the diffraction limit of light microscopy with the advent of EM. In this work, for the first time, we propose to use the TEM EDX method for imaging biomolecules using mAb labeled with MNPs of complex iron oxides. The binding of mAb to protein antigens will allow selective delivery of MNPs containing metal cations in the crystal lattice to the target, while TEM with energy dispersive analysis will allow their localization to be determined with resolution up to few nm, thus providing efficient visualization of mAb binding to protein antigen with a resolution exceeding the diffraction limit of light microscopy.

The main goal of the present work. The aim of this work is to create conjugates of MNPs based on complex iron oxides with different divalent cations with mAb for visualization of protein molecules in cellular structures by TEM. To achieve the goal, the following tasks were formulated and solved:

1. Development of methods for the synthesis of complex iron oxides such as Fe_3O_4, $MnFe_2O_4$, $CoFe_2O_4$, $ZnFe_2O_4$ with the size < 20 nm, with the elemental ratio of metal cations Mn^{2+}, Co^{2+},Zn^{2+}:Fe^{3+} as close as possible to 1:2;
2. Development of functional coatings, allowing to obtain stable colloidal suspensions of MNPs carrying functional groups for modification with mAbs;
3. Investigation of the immunochemical activity of mAb after conjugation with modified Fe_3O_4, $MnFe_2O_4$, $CoFe_2O_4$, and $ZnFe_2O_4$ MNPs;
4. To show the possibility of visualization of biomolecules in cellular structures using conjugates of MNPs with mAb by TEM.

Scientific Novelty. An original methodology for the synthesis of MFe_2O_4 (M=Mn, Co, Zn) MNPs in benzyl alcohol was developed, and the influence of benzyl alcohol and benzyl ether with oleic acid on the elemental composition of the obtained MNPs was investigated. It was shown that the use of DOPAC and PEG-COOH molecules for functionalization of the surface of MNPs makes it possible to obtain stable aqueous colloidal solutions of MNPs that allow conjugation with mAb_{II} with preservation of their immunochemical activity. Conjugates of MNP with mAb_{II} have been shown to be able to bind to protein antigens in cellular compartments and can be visualized by TEM. In addition, for the first time, EDX analysis in tandem with HAADF STEM was performed, allowing the high-resolution detection of single $CoFe_2O_4$ MNPs associated with protein antigen presented in mitochondria, and the cationic composition of such MNPs could be reliably identified.

Theoretical and practical significance of the work. The simplicity and adaptability of the developed experimental synthesis procedure for the preparation of complex iron oxides of controlled elemental composition in this thesis work provides practical recommendations for a wide range of applications (RF Patent №2787203, 2022). The developed method of visualization of biological objects, combining electron microscopy with EDX analysis, using nanoprobes based on MNPs of complex iron oxides, will make it possible to detect several targets at once, where each type of atoms can be mapped with a certain color, increasing the resolution to ultra-high values (1-3 nm) (NUST MISIS know-how technology № 05-645-2020 at December 2, 2020 for "Method of simultaneous visualization of biological structures").

Defense provisions:

1. The developed methods for the synthesis of complex iron oxide MNPs make it possible to obtain monodisperse MNPs with a strictly defined stoichiometric composition and a diameter from 3 to 10 nm.

2. The stoichiometric composition and the size of the magnetic nucleus can be controlled by selecting the reaction parameters.

3. The modification of the MNPs surface with DOPAC and PEG-COOH molecules provides aggregative stability of the MNPs structure in water-salt buffers and allows fixation of mAb on its surface.

4. The use of carbodiimide method for binding of MNPs to mAb allows to preserve the immunochemical activity of mAb in the developed conjugate, which is a key parameter determining their specificity towards the selected target.

5. It has been shown that conjugates of MNPs with mAb are able to bind to protein antigens in cellular structures, and can be visualized by TEM.