Functionalized Fluorescent Nanoparticles for Cell Labeling for Further Sensor Diagnostics

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***Abstract* — Core size of the core and the nature of semiconductor CdSe/ZnS nanoparticles (NP's) were the subject of the research to understand main reasons of the dynamics of "living cell/nanoparticle" interactions. Core size of NP (with a given type of covering) has a significant impact on their uptake by cells. Nanoparticles with core size 1.7 nm entrapped efficiently when cysteine and mercasptoundecanoic acid are used as coatings. However, the factor of the size of NP's is not always determinable. Indeed, NP's coated with cysteamine were captured by cells to a small extent onto the contrary to fluorescent nanoparticles covered with cysteine and mercaptoundecanoic acd. Additional modification of NP shell with antibody allows labeling of target cells that open perspectives for disease diagnostics.**

***Index Terms* — nanoparticle, fluorescence, diagnostics, cell labeling, cell.**

I. INTRODUCTION

Inorganic semiconductor fluorescent particles attract great attention in medicine and biology due to unique spectral properties: they absorb light in broad spectral range, have narrow symmetric emission spectra, possess a high fluorescence quantum yield and a large Stokes shift [1, 2]. At present different types of nanoparticles (NP's) are already applied for labeling and modulation of cell functions, namely for non-invasive imaging of cells and tissues both *in vitro* and *in vivo*. Particle uptake by cells allows one to use them as a fluorescent label for specific cell populations, including tumor cells. The nature of the shell that stabilizes a core of nanoparticles is extremely important for specific targeting. In particular, a coating based on polyethylene glycol (PEG) is the most widely used among the shells onto NP surface that blocks non-specific binding [3, 4]. Receptor molecules such as antibodies (AB) are used as receptor molecules on outer shell of nanoparticles. It is possible to label nanoparticles with such receptors and visualize the target cells owing to "antigen-antibody" interactions [5]. Therefore, the methods for isolating and recording "cell fluorescent nanoparticle" complexes ought to be used in medicine for diagnosis of various diseases, including oncology, bacteriology, and virology. It is extremely important to study the nature of the shell of nanoparticles on their cellular recognition. That is why the goal of our research is to elaborate the methods of modification of surface of nanoparticles with different shells and to understand the main reasons of the dynamics of cell-nanoparticle interactions.

II. EXPERIMENTAL PART

Water-soluble CdSe/ZnS NP's were used. Nanoparticles (cadmium selenide quantum dots coated with zinc sulfide) that is synthesized according to [6, 7] having a size of 1.7, 2.4 and 2.8 nm respectively. Hydrophobic shell of TOPO (trioctulphosphin oxide) was deleted by changing solvents and stabilized organic ligands. Each type of quantum dot was modified by three types of organic shells: mercaptoundecanoic acid (MUA), cysteamine (CA), and cysteine (Cys), Fig. 1.

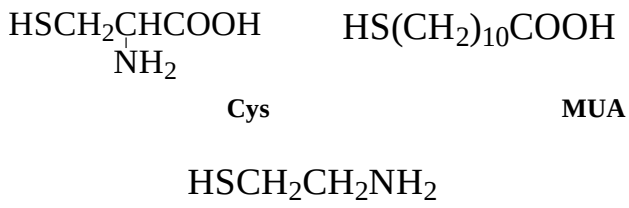

Fig. 1. Chemical structures of stabilizing ligands

Immune cells were obtained from AF line of mice, tumor-bearing solid Ehrlich carcinoma at 14–21 days after subcutaneous inoculation.

The animals were euthanized by cervical dislocation. The cells were obtained from peritoneal washout, homogenates of spleen and lymph nodes, and from bone marrow suspensions. Cells were washed with a solution of Earl and supernatant was removed and the cells were re-suspended in a solution of Earle. Hypotonic shock lysis was carried out after the first washing in the case of the presence of significant admixture of red blood cells in the samples (re-suspension of precipitate in distilled water, and restoration of isotonicity by sodium chloride solution 40 seconds later), and the cells were washed with Earl solution after that. Cell populations of the immune system of mice were obtained with the viability of at least 85%, heterogeneous both in origin and functions. All populations of cells contained macrophages, which were proved morphologically. The maximum amount of

macrophages, which was found in the peritoneal washings, ranged from 20 to 50%.

Distilled water, the suspension of NP's, hypertonic solution of sodium chloride, and the cell suspension were added to the holes of a 96-socket plate to a final concentration of the nanoparticles equal to 0.33, 0.1 and 0.05 mg/ml.

Cells were incubated with nanoparticles in a moist chamber at 37 ºC for 2–3 hours to estimate the entrapment and 5 hours - to estimate the cytotoxicity. As a negative control, cells were incubated under similar conditions in the absence of NP.

A fluorescence microscope «Leica» was used.

For fluorescence quenching of unabsorbed NP and labeling of dead cells, trypan blue dye was used.

III. RESULTS AND DISCUSSION

Three types of nanoparticles with different sizes of the core were studied. It is shown that the fluorescence intensity of the nanoparticles decreases in the range from small NP's to big ones. Figure 2 shows the emission spectra of the cysteine-stabilized NP's in distilled water at a concentration of 0.01 mg/ml.

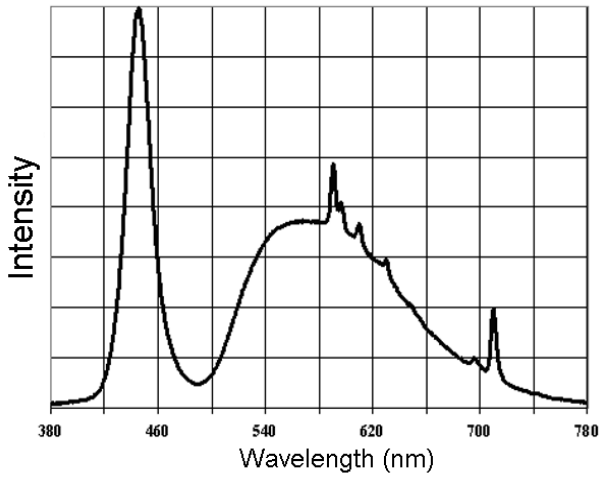

Fig. 2. The fluorescence spectra of CdSe/ZnS NP's with different size of core covered with cysteine shell.

MUA- and Cys-stabilized NP's were captured primarily by macrophages that present in all cell populations. Cysteamine-stabilized NP's formed aggregates and only slightly absorbed by cells.

There were no significant differences in the entrapment of NP's by cells of various tissue origins. The optimum concentration of NP's for absorption and estimation of the cytotoxicity was 0.1 mg/ml, since the concentration of 0.33 mg/ml showed toxicity of NP, and worse registered at a concentration of 0.05 mg/ml.

NP's with a core diameter of 1.7 nm are captured by phagocytes less than in an hour. The NP's have been localized in sub-membrane vesicles, presumably in the phagosomes, after 3 hours.

For phagocytosis of the larger (diameter of 2.4 and 2.8 nm) nanoparticles require a longer incubation time – for at least 2–3 hours. All types of the particles in some degree captured by macrophages with longer (3–5 hours) incubation. It is established that the cytotoxic effect of suspensions of NP's developed after 3–5 hours of incubation. Thus, cysteine stabilized NP, with the core size of 1.7 nm reduced cell viability by 12% (5.5–21.5%), with core size 2.4 nm and 2.8 nm at 13% (5–18%) and 11.5 (3–23%) as compared to the control respectively. Cysteine-coated nanoparticles with a core of different sizes did not differ in cytotoxicity (Table 1).

Table 1: The vitality decrease of immune cells in the presence of NP's as compared with control, median (25-75%)

The diameter of a core, nm	Covering		
	MUA	Cystein	Cysteamin
1.7	30% (7–33.5%)*	12% (5.5–21.5%)*	n.s.
2	30% (7–33.5%)*	12% (5.5–21,5%)*	n.s.
2.4	35% (25–67%)*	13% (5–18%)*	11% *

* $p < 0.05$ as compared with control.

MUA–stabilized NP's are more toxic compared to cysteine coated NP's, which is the reason of the reduction in cell viability by 30% (7–33.5%), 35% (25–67%), and 33.5 (19.4–51%) in the presence of nanoparticles with core diameter between 1.7 and 2.8 nm, respectively. Here, also found no effect of the core size on the toxicity of the particles. NP's coated with cysteamine, with core size 1.7 nm showed no significant toxicity, while the nanoparticles with core 2.4 nm and 2.8 nm in some cases cause significant decrease in cell viability of peritoneal cavity and lymph nodes at 11 and 34 % respectively. Dependencies between the ability of penetration of NP's into cells and their toxicity was not observed.

Not only phagocytic cells can entrap fluorescent nanoparticles as it was established by studying the interaction of nanoparticles with different stabilizing shell with different cell cultures, Figs. 3–5.

It was also found that fluorescent nanoparticles with cysteine shell can penetrate inside the cell membrane and accumulate in certain areas of mesenchymal stem cells, Fig. 4. Keeping in mind the small size of the nanoparticles, it is obvious that the bright spots recorded by fluorescence microscopy, in fact, are aggregates of many nanoparticles.

On the contrary, NP's adsorb only on the outer shell of cell membrane in the case of cancer cells regardless of the type of coating on fluorescent nanoparticles, Fig 5.

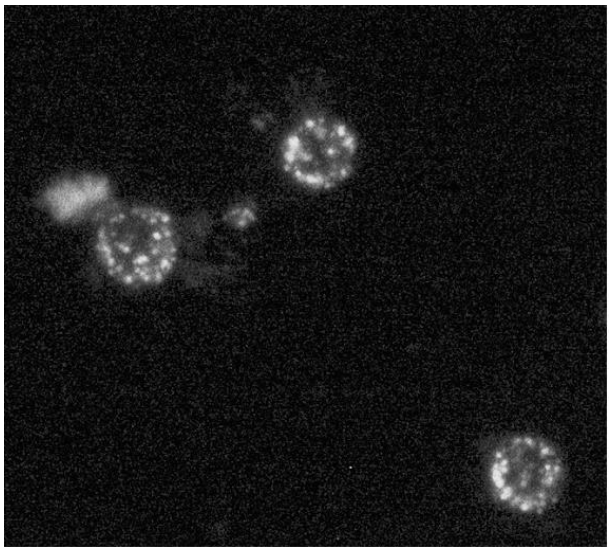

Fig. 3. Fluorescent image of phagocytic cells with captured CdSe/ZnS/Cys nanoparticles

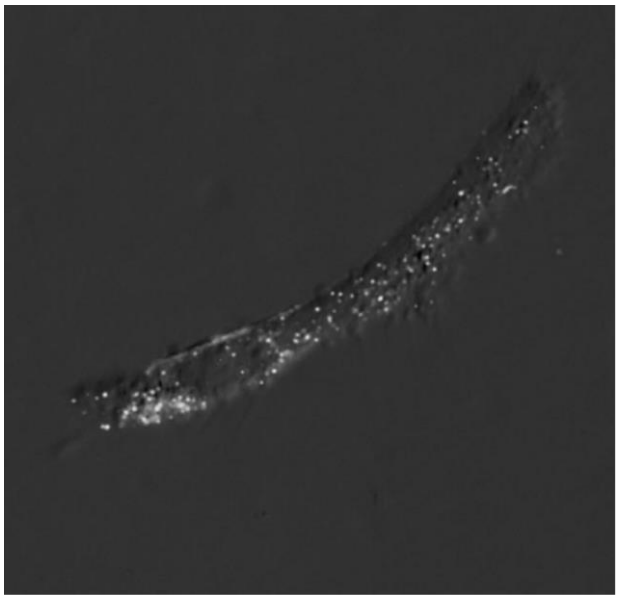

Fig. 4. Fluorescent image of mesenchymal stem cell labeled with CdSe/ZnS/Cys nanoparticles.

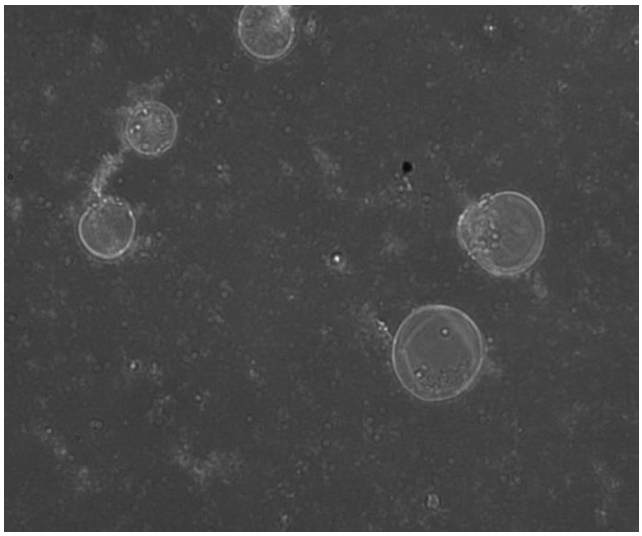

Fig. 5. Fluorescent image of cancer cells of carcinoma Ehrlich painted by CdSe/ZnS nanoparticles with stabilizing shell of mercaptoundecanic acid.

Subsequent NP's modification with such receptor molecules as antibodies [5] has lead to target cell labeling, Fig. 6.

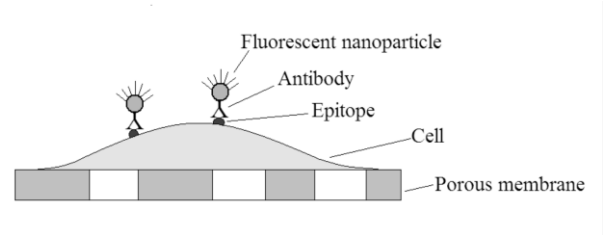

Fig. 6. Schematics of cell that marked with fluorescent nanoparticles.

For example, selective recognition of McCoy cells that infected by *Chlamydia trachomatis* was done using NP's modified by specific antibodies, Fig. 7.

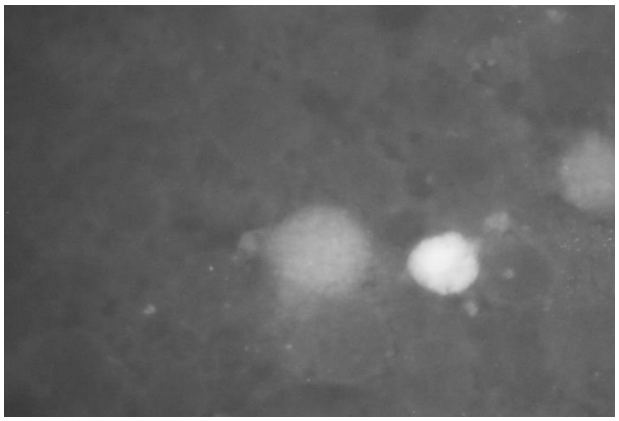

Fig. 7. Cell population of McCoy cells after treatment with CdSe/ZnS/AB marker: bright illuminated cells are infected by *Chlamydia trachomatis*

The registration system for fluorescent analysis of labeled cell population is under construction. The experimental results allowed us to develop the project of the system of detection of biological molecules.

IV. CONCLUSIONS

Core size of NP's (with a given type of covering) has a significant impact on their uptake by cells. Nanoparticles with core size 1.7 nm were entrapped efficiently when cysteine and mercasptoundecanoic acid are used as coatings. However, the factor of the size of NP is not always determinable, as it was confirmed experimentally. Indeed, NP's coated with cysteamine were captured by cells to a small extent onto the contrary to fluorescent nanoparticles covered with cysteine and mercaptoundecanoic acd.

In contrast to the entrapment of nanoparticles, no relation has been revealed between the degree of cytotoxicity of NP *in vitro* and their size.

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