LUMINESCENT QUANTUM DOTS ENCAPSULATED BY ZWITTERIONIC AMPHIPHILIC POLYMER: CALCIUM-DEPENDENT INTERACTION WITH CELLS

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Luminescent semiconductor nanocrystals known as quantum dots (QDs) are found applications in various biological assays: bioimaging, cell functioning, and intercellular interactions [1]. Unlike organic dyes QDs possess high photostability and photoluminescence (PL) quantum yield (QY), broad absorption range and narrow emission peaks. These parameters make QDs as perspective alternative for traditional organic fluorophores. Monodisperse colloidal QDs with proper optical characteristics are made by high-temperature organic synthesis resulting in QDs with a hydrophobic surface ligand layer. For biological environments, post-synthetical treatment is needed. Modification of surface with an amphiphilic polymer (poly(maleic anhydride alt-1-tetradecene), PMAT) leads to NCs with small hydrodynamic size and preserved QY [2]. Derivatization of anhydride group of PMAT with different ionic groups improves biocompatibility and changes physical-chemical characteristics: zeta-potential and size. Surface charge influences the effectiveness and selectivity of interactions of QDs with cell cultures because the initial contact NCs and cells is determined by attractive forces such as electrostatic, hydrophilic, hydrophobic, etc. [3]

The aim of this work was to study the impact of zeta-potential and Ca^{2+}-ions on the effectiveness of cell labeling. Ca^{2+}-ions were selected due to their importance in a number of cell processes (signal transduction pathways, blood-clotting) and influence on zeta-potential QDs markers.

Chemical modification of PMAT was performed according to the procedure that involved a reaction of PMAT in organic solution with (2-aminoethyl)trimethylammonium chloride. The ratio of quaternary ammonium and carboxylic groups determines the surface charge of encapsulated nanoparticles. After chemical modification of PMAT hydrophobic CdSe/ZnS core-shell QDs were encapsulated by modified PMAT according to the standard protocol. Encapsulated QDs in a dry solid form were dissolved in phosphate buffer solution, zeta potential and hydrodynamic size of water-soluble QDs were measured with the dynamic light scattering analyzer Malvern Zetasizer NanoZS90. The labeling procedure was performed by mixing the suspension of cell culture and NCs solution at fixed pH, buffer and Ca^{2+}-ion concentration. Then cells were washed with phosphate buffer and labeled cells were explored with BD FACSCanto II Flow Cytometer and analyzed by fluorescence microscopy.

It was found that cells are labeled the most effective with NCs of low positive zeta-potential. However, the addition of Ca^{2+}-ions leads to decrease in PL intensity by a factor of two. This effect can be explained by the increase of zeta-potential and less intense cell binding, as well as the ability of Ca^{2+}-ions to quench the PL.

Table 1. Dependence of PL intensity of labeled cellular culture rat C6 glioma versus NCs zeta-potential and Ca^{2+}-ions presence.

Sample	Zeta-potential, mV	PL intensity, a.u.	Zeta-potential after Ca^{2+} adding, mV	PL intensity after Ca^{2+} adding, a.u.
1 (0% modified)	-33	0.3	-15	1.6
2 (50% modified)	+13	2.3	+27	1.1
3 (100% modified)	+23	1.6	+27	1.7

The presence of Ca^{2+}- ions increases the fluorescence of cells if negatively-charged NCs are used. As shown by microscopic analysis, in the presence of Ca^{2+}- ions the nanoparticles are deposited on the cell membrane, forming large agglomerates. This phenomenon can be explained binding of carboxylic groups and calcium ions on the cell membrane. It is also possible that the shift of zeta potential to the positive side also plays a role in enhancing cell labeling.

Labeling with positively-charged NCs are not influenced by calcium addition due to electrostatic repulsion of quaternary ammonia groups and Ca^{2+}-ions.

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[1] N. Tomczak, R. Liu, J. G. Vansco, Nanoscale **5**, 12018 (2013).
[2] Pellegrino, T.; Manna, L.; Kudera, S.; Liedl, T.; Koktysh, D.; Rogach, A. L.; Keller, S.; Rädler, J.; Natile, G.; Parak, W. J. Nano Lett. **4**, 703 (2004).
[3] E. A. Petrova, T. I. Terpinskaya, A. A. Fedosyuk, A. V. Radchanka, A. V. Antanovich, A. V. Prudnikau, M. V. Artemyev, Journal of Belarusian State University 2, 3 (2018).