Cell Toxicity and Preparation of Streptavidin-Modified Iron Nanoparticles and Glutathione-Modified Cadmium-Based Quantum Dots

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Abstract

This paper is focused on the investigation of conjugate nanostructures consisting of superparamagnetic $\gamma$-Fe$_2$O$_3$ nanoparticles (NPs) and CdSe/CdS quantum dots (QDs) with core/shell structure. In the first step, we prepared streptavidin (STP) conjugated Fe$_2$O$_3$ magnetic particles. The next stage was preparation of QDs using method based on surface modification of hydrophobic core/shell CdSe/CdS with biotinylated glutathione. Glutathione (GSH) belongs to the most abundant peptides in organisms maintaining redox status, which, from the “particle” point of view, makes the QDs water-soluble and stabilized in aqueous solution. Biotin molecule is known for its excellent affinity to streptavidin and hence allows the conjugation of both nanostructures (magnetic and light-emitting). We tested the influence of both prepared nanostructures on growth and viability of fibroblasts and BY-2 tobacco cells, respectively. NPs-STP and QDs-GSH may not pose a threat to cells, but non-modified nanoparticles and QDs without GSH had a very adverse effect on cells.

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1. Introduction

Magnetic nanoparticles (MNPs) are being of great interest due to their unique purposes. Especially in medicine and pharmaceutics, application of MNPs is much promising. \cite{1} The unique combination of high magnetization and paramagnetic or superparamagnetic behavior opens this material to a very large scale of application. Particularly, the
possibilities of their modification by biologically active compounds are very interesting using them in controlled drug delivery systems, as agents in magnetic resonance imaging and for magnetic-induced tumor treatment via hyperthermia [2]. Similarly, quantum dots (QDs) are also extensively investigated due to its application medicinal and pharmaceutical industries, mainly for in vivo and in vitro imaging [3]. Semiconductor nanocrystals have size-tunable emissions due to quantum size effects and exhibit high resistance toward photobleaching.

The conjugate nanostructures consisting of superparamagnetic NPs and QDs give significant importance as a new class of multifunctional nanoscale structures, particularly in their adaptability for biolabeling/imaging and cell sorting/separation. In these conjugate nanostructures, the magnetic NPs may serve as transport agent that can be manipulated by an external magnetic field gradient, and the QDs may act as fluorescent tags, therefore allows an optical window for in vitro cell detection. Furthermore, the conjugate structures can be functionalized with biorecognition molecules that would be selectively delivered to a biological target, and hence would be compatible with personal medical applications. The magnetic-semiconductor conjugate can also be equipped with drug molecules to be released at the specific site of the cancer cells, for the simultaneous targeted therapy of the labeled target [4].

2. Result and discussion

2.1. Experimental design

Experimental design is shown in Fig. 1. Paramagnetic particles are modified with streptavidin with covalently bound biotin-GSH (biotin-reduced glutathione) and quantum dots (CdSe/CdS) [5].

Fig. 1 Experimental scheme of paramagnetic particles modification with streptavidin with covalently bound biotin-GSH (biotin-reduced glutathione) and quantum dots (CdSe/CdS). The modified particles give fluorescent signal.

2.2. Preparation and characterization of modified particles

In this paper we presented a method for the preparation of γ-Fe₂O₃ NPs functionalized with STP and glutathione-coated QDs (CdSe/CdS) with core-shell structure. The phase analysis of Fe₂O₃ NPs carried out from the Mössbauer spectroscopy as well as XRD analysis was detailed discussed in our previous work [6]. After it, the surface of hydrophobic CdSe/CdS QDs was modified with biotinylated glutathione (GSH). In number of papers, there are commonly used biotinylation peptides, proteins and nucleic acids. However, binding of biotin to the low molecular weight thiols is not easy. Under our experimental conditions, we succeeded and the presence of biotin-GSH modification was verified by mass spectrometry. The peak at mass 532 m/z (biotin-GSH) was well detectable. Besides the thiol group served as capping agent, each GSH molecule also contains one amine and two carboxylate groups. We found out these functional groups provide the possibility of being coupled and further crosslinked to
form a polymerized structure [7]. Our prepared CdSe/CdS QDs give a green fluorescence with maximum at 508 nm (Fig. 2). Coupling of paramagnetic particle and quantum dots (as a tag) brings many advantages including isolation capabilities and sensitive detection [8-11].

In addition to the experiment with the intensity of fluorescence of quantum dots coupled to GSH, we focused on the electrochemical detection of biotin-GSH. The bound between biotin-GSH and streptavidin-modified particles was broken at 99 °C and low pH. Free biotin-GSH was detected by differential pulse voltammetry. We were able to demonstrate that the surface modified nanoparticles with streptavidin binds glutathione, depending on the applied concentration.

Fig. 2 Fluorescence spectrum of CdSe/CdS QDs in THF (black) and CdSe/CdS QDs modified with biotinylated glutathione (red) compared with biotinylated glutathione solution (green, B). Green fluorescence of irradiated QDs (A).

2.3. Study of QDs influence on BY-2 tobacco cells

BY-2 tobacco cells are widely used as model for studying of biochemical, transporting and signaling processes by plant physiologist, molecular biologists and many other scientists from various branches. BY-2 tobacco cell culture has been also proved as useful tool for analysis of plant cell cycle because of very easy synchronization of their cell cycle. BY-2 tobacco cells can be also easily analyzed using microscopic techniques, hence, numerous protocols have been established for studying of cell structure and individual cell organelles on this culture. Therefore, BY-2 cells, thank to their characteristics, are comparable with HeLa cells for human research [12]. In this study, the cells were treated with 0, 10 and 100 μM of cadmium(II) ions. It is known that cadmium(II) ions significantly decrease cell viability and, moreover, cause condensation of chromatin on the periphery of nucleus and around nucleolus and DNA fragmentation with formation of apoptotic-like bodies and cytoplasm shrinkage. We investigated the influence of these ions by using QDs. The untreated BY-2 tobacco cells accumulated QDs on the periphery of cells in cytoplasm. With increasing Cd(II) ions concentration and time of the treatment, QDs were accumulated in cytoplasm, especially around nucleus. Distribution of QDs into vacuoles has not been demonstrated.

In the proximity to nucleus, single-membrane organelles, such as Golgi and endoplasmatic reticulum, are localized. These organelles probably play important role in the processes of calcium-induced apoptosis, which has been described in the case of treatment of cells by Cd(II) ions. Cd(II) ions are also able to induce reactive oxygen species (ROS), which induce processes of programmed cell death [13]. Therefore, it is possible that QDs containing glutathione molecules, which plays important role in heavy metals ions detoxification, are concentrated in the proximity to these two organelles playing the important role in heavy metals ions detoxification.
3. Conclusion

The prepared GSH modified CdSe/CdS quantum dots have been utilized for investigation of cadmium(II) ions toxicity on BY-2 tobacco cells. QDs, which have not been modified with GSH, influenced tobacco cells extremely toxically. Their using in vivo is completely inappropriate. QDs modification dramatically reduces toxicity. In addition to the toxicity test, we investigated the effect of cadmium(II) ions on cell viability. Cadmium(II) ions significantly reduced cell viability due to many adverse effects including production of ROS, however, the presence of QDs should be a new way to study the effects of these ions on cells.

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References