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Effects of silver sulfide quantum dots coated with 2-mercaptopropionic acid on genotoxic and apoptotic pathways *in vitro*

Deniz Ozkan Vardar[a], Sevtap Aydin[b], Ibrahim Hocaoglu[c], Funda Havva Yagci Acar[d], Nursen Basaran[b,*]

[a] Hitit University, Sungurlu Vocational High School, Health Programs, Sungurlu, Çorum, Turkey
[b] Hacettepe University, Faculty of Pharmacy, Department of Toxicology, Ankara, Turkey
[c] TÜBİTAK Marmara Research Center, Genetic Engineering and Biotechnology Institute, Istanbul, Turkey
[d] Koc University, Department of Chemistry, Istanbul, Turkey

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ABSTRACT

Quantum dots (QDs) are highly promising nanomaterials in bioimaging system because of their bright fluorescence, broad UV excitation, narrow emission band, and high photostability. Recently, there is a great activity on Ag_2S quantum dots for both imaging and drug/gene delivery due to the potential of having a better cytocompatability and near infrared luminescence. 2-Mercaptopropionic acid (2 MP A)-coated silver sulfide (Ag_2S) QDs were reported as the most luminescent, stable, anionic Ag_2S QDs in the literature. In this study, we aim to determine the cytotoxicity of 2 MP A/Ag_2S in Chinese hamster lung fibroblast (V79) cells. The genotoxic and apoptotic effects of 2 MP A/Ag_2S QDs were assessed by the alkaline single cell electrophoresis assay and real time polymerase chain reaction techniques, respectively. The cell viability decreased above 200 μg/ml and 800 μg/ml for MTT tetrazolium and neural red uptake assays, respectively. DNA damage was not observed by 2 MP A/Ag_2S QDs at the studied concentration levels (5–2000 μg/ml). The levels of mRNA expression of p53, caspase 3, caspase 9, bax, bcl-2, survivin were not changed by 2 MP A/Ag_2S QDs below IC50 (around 1000 μg/ml). Hence, 2 MP A/Ag_2S QDs did not show any cytotoxic or genotoxic effects in V79 cells at lower doses. We conclude that the biocompatibility of 2 MP A/Ag_2SODs makes them suitable for cell labeling applications.

1. Introduction

Nanotechnology is an emerging field that involves the manufacturing and measurement of materials and systems in the nanometer range [1]. The reduction of material dimensions to the nanoscale is known to alter many physicochemical properties and these prominent characteristics may be structural, chemical, optical, electrical, and magnetic. These properties interact with biological systems in an unprecedented manner. Nanoparticles have unique features such as high surface-to-volume ratio, surface curvature, and high surface reactivity. Besides, they may be produced in different size, chemical composition, shape and surface charge which affect their passage across the cell membrane, biodistribution, and toxicity [2,3]. Recently, the use of nanomaterials (NMs) has attracted great interest in the biomedical field [4].

Quantum dots (QDs), nano sized semiconductor crystals, are comprised of groups II-VI or III-V elements, and described as 'synthetic atoms'. Their energy levels are specific, and the changes in their dimensions can modulate their bandgap [5]. Due to the fact that QDs have high optical stability, broad absorption and narrow emission spectra, they have specific electronic features and luminescence specifications [6]. Photoexcitation of QDs generate long lived, strong luminescence. The wavelength of luminescence can be tailored with the size and/or composition of the QDs. Besides, many QDs are able to be excited with a single excitation source, which results in efficient multiplexing and concurrently detection of a multitude of markers in a single specimen [7,8]. Hence, they are great alternatives to fast quenching organic fluorophores which have specific excitation wavelengths. QDs, being mostly used in staining fixed cells and tissues, or in *in vivo* imaging, have precious fluorophores in the biomedical field by virtue of their fluorescent features [8,9]. They interact with the biological sciences, which give a great chance to find a way of QDs into several commercial consumers and clinical products [10].

QDs are usually synthesized with II–VI materials such as ZnS, CdS [11,12]. Structurally, QDs have a metalloid crystalline core and a "cap or shell" that covers and protects the core and makes the QD

* Corresponding author.
E-mail address: nbasaran@hacettepe.edu.tr (N. Basaran).

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bioavailable. These cores can be made from different materials with different band gaps for luminescence in the visible or near infrared region (NIR). For instance, Cd or Zn chalcogenides such as CdS, CdSe, CdTe, ZnS are examples to group II–VI series [13,14] with luminescence in the visible optical window while indium phosphate and indium arsenate are examples to group III–V series with emission from red to NI, and PbS and PbSe are examples to QDs with emission in the NI. There are also core/shell QDs such as CdTe/CdSe, CdSe/ZnTe [15].

The major limitation for the clinical use of QDs are potential toxicity due to their chemical composition and nanoscale features [15]. The most known QDs for biomedical applications have been recently based on CdSe core materials showing the greatest quality materials that exhibit the most control over nanocrystal spectroscopic characteristics. Although there are many studies on non-toxic compositions in some degree delivered to cells, the disquietudes with respect of the cytotoxicity of released cadmium ions and the associated oxidative stress have been unsolved [16–19] The exact mechanisms of toxicity of nanoparticles are not well understood, but their genotoxic and apoptotic effects are frequently reported [3,20–22]. Nanoparticle toxicity may be related to redox imbalance which leads to major oxidative damages to DNA via oxidative stress.

Within the last decade, there is a tremendous effort in developing Cd-free quantum dots. In addition, considering *in vivo* optical imaging potential, luminescence in the NI, most specifically 700–900 nm is desired to suppress the auto-fluorescence of the biological constituents and to provide deeper tissue penetration. Ag_2S QDs emerged recently as new generation QDs satisfy both of these criteria [23,24]. 2-Mercaptopropionic acid-coated silver sulfide (2 MP A/Ag_2S) are strongly luminescent, anionic, NI-emitting properties [24]. These particles were internalized significantly by NIH/3T3 cells and provided strong intracellular optical signal, suppressing the autofluorescence. No reduction in the viability of NIH/3T3 cells up to 600 μg/ml was reported, which is quite unusual for a non-PEGylated QD. As the surface carboxylic acids is conjugated with target ligands or drugs, this composition is of particular importance for many applications, thus producing therapeutic nanoparticles. However, there exists a need for more detailed toxicity analysis of such particles. The data presented here is the first data that gives a cytotoxic, genotoxic and apoptotic effects of 2 MP A/Ag_2S QDs *in vitro*.

In this study, we aimed to investigate the ctotoxicity, genotoxicity and apoptosis caused by 2 MP A/Ag_2S QDs in Chinese hamster lung fibroblast (V79) cells to clarify the mechanisms underlying its potential toxicity.

In short, in order to have a more comprehensive toxicity analysis of 2 MP A/Ag_2S QDs, we tested the viability of cells by two different assays; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and neutral red uptake (NRU) assays. We investigated potential genotoxicity by comet assay and determined the regulation of apoptotic genes by the real time polymerase chain reaction (RT-PCR) technique.

2. Materials and methods

2.1. Chemicals

The chemicals were purchased from the following suppliers: hydrogen peroxide (35%) (H_2O_2) from Merck Chemicals (Darmstadt, Germany), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), acetic acid, dimethyl sulfoxide (DMSO), DMSA, *Dublecco's* modified eagle's medium (DMEM), ethanol, ethidium bromide (EtBr), fetal bovine serum (FBS), low melting point agarose (LMA), L-glutamin, neutral red (NR), sodium chloride (NaCl), sodium hydroxide (NaOH), N-lauroyl sarcosinate, normal melting point agarose (NMA), Silver nitrate ($AgNO_3$), trypsin-EDTA, triton X-100, penicillin-streptomycin, phosphate buffered saline (PBS), from Sigma-Aldrich Chemicals (St. Louis, MO, USA). Sodium sulfide (Na_2S) was purchased from Alfa-Aesar (Thermo Fisher Scientific, Karlsruhe, Germany). Milli-Q water (18.2 MOhm) was used as the reaction medium.

2.2. Preparation and characterization of 2 MP A/Ag_2S NIRQDs

2 MP A/Ag_2S NIR QDs were prepared in a one-step reaction. A detailed description and characterization of 2 MP A/Ag_2S QDs designed as an original particle were performed previously by Hocaoglu et al. [24]. Briefly, 2-MPA (1.25 mmol) was dissolved in 375 ml of deoxygenated deionized water and the pH was adjusted to 7.5 b < superscript > < / superscript > y using NaOH and CH_3COOH solutions (2 M). $AgNO_3$ (0.25 mmol) was added and the pH was readjusted to 7.50, again. Reaction mixture was stirred at room temperature for 5 h. 125 ml of deoxygenated aqueous Na_2S (0.0625 mmol) solution was then added to the reaction mixture under vigorous stirring. Colloidal stable 2 MP A/Ag_2S QDs were washed with deionized water using Amicon-Ultra centrifugal filters (3000 Da cut off) and stored in dark at 4 °C.

In order to calculate the QDs concentration few ml of the colloidal solution was dried in freeze-drier. The concentration of the QD solution was determined as 4.6 mg/ml. Absorbance spectrum of QDs was taken with a Shimadzu 3101 PC UV-vis-NIR spectrometer in the 300–1000 nm range. Photoluminescence spectrum was obtained using a home-made spectrophotometer as described in detail previously by Hocaoglu et al. [24]. Samples were excited with a continuous-wave, frequency-doubled Nd: vanadate laser operating at 537 nm and emission was recorded by an amplified silicon detector in the range of 400–1100 nm with a lock-in amplifier. Particles absorb strongly until 850 nm and emit in the NIR with a peak maxima at 870 nm when excited at 537 nm. Malvern zeta sizer nano S (red badge) ZEN 1600 was used for the measurement of hydrodynamic size (3.0 nm) of aqueous QDs. The zeta potential of aqueous QDs as −10 mV due to the anionic coating was measured with Brookhaven zetapals, Zeta potential analyzer instrument by using the Smoluchowsky model.

The diameter of this Ag_2S was calculated as 2.66 nm using the Brus equation [24]. A representative transmission electron microscopy (TEM) image of Ag2S-2MPA QDs shows a spherical shape (Fig. 1). We measured the hydrodynamic size and zeta potential at 200 μg/ml dose. Medium used in our experiement caused some agglomoration along with the changes in zeta potential as would be expected due to the interaction of the medium components with nanoparticle. For Ag_2S-2MPA QDs in PBS, the size (number) was 3.74 ± 0.33; pdi was

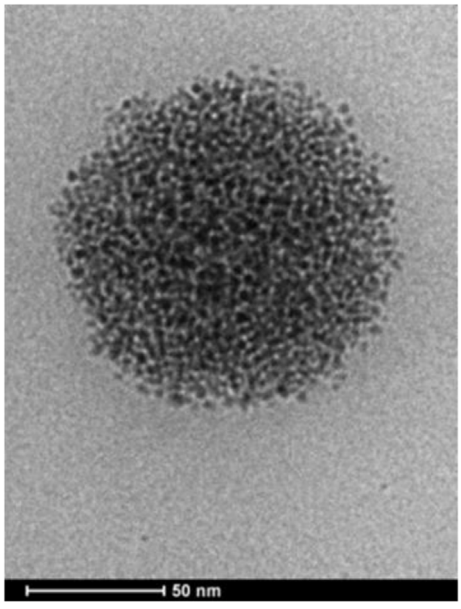

Fig. 1. TEM images of 2 MP A/Ag_2S QDs. Scale bar: 50 nm. TEM, transmission electron microscopy.