Materials Science and Engineering C 69 (2016) 52–59

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Influence of heavy nanocrystals on spermatozoa and fertility of mammals

Omid Akhavan [a,b,*], Ehsan Hashemi [c], Hakimeh Zare [d], Mehdi Shamsara [c], Nima Taghavinia [a,b], Farid Heidari [c]

[a] Department of Physics, Sharif University of Technology, P.O. Box 11155-9161, Tehran, Iran
[b] Institute for Nanoscience and Nanotechnology, Sharif University of Technology, P.O. Box 14588-89694, Tehran, Iran
[c] National Research Center for Transgenic Mouse, National Institute of Genetic Engineering and Biotechnology, P.O. Box 14965-161, Tehran, Iran
[d] Physics Department, Yazd University, Yazd, P.O. Box 89195-741, Iran

ARTICLE INFO

Article history:
Received 13 February 2016
Received in revised form 7 June 2016
Accepted 16 June 2016
Available online 16 June 2016

Keywords:
Quantum dots
CdTe
Toxicity
Sperm
Fertility
Mice

ABSTRACT

In recent years, quantum dots (QDs) have been widely used in upcoming nanotechnology-based solar cells, light-emitting diodes and even bioimaging, due to their tunable optical properties and excellent quantum yields. But, such nanostructures are currently constituted by heavy elements which can threat the human health and living environment. Hence, in this work, the *in vivo* effects of CdTe nanocrystals (NCs) (as one of the promising QDs) on spermatozoa of male mice and subsequently on fertility of female mice were investigated, for the first time. To do this, CdTe NCs were synthesized through an environment-friendly (aqueous-based solution) method. The sperm cells presented a high potential for uptake of the heavy QDs. Meantime, the NCs exhibited concentration-dependent adverse effects on morphology, viability, kinetic characteristics and DNA of the spermatozoa. At low concentration of 0.1 μg/mL, the NCs showed a moderate toxicity (~25% reduction in viability and motility of the spermatozoa), while remarkable toxicities were observed at higher concentrations of 1.0–100 μg/mL (~67% reduction in viability and motility for 100 μg/mL). Furthermore, significant *in vitro* DNA fragmentation of the spermatozoa was observed at CdTe concentrations ≥10 μg/mL. *In vivo* toxicity of the NCs was found lower than the *in vitro* toxicity. Nevertheless, the *in vivo* destructive effects of the NCs still caused ~34% reduction in viability as well as motility and ~5% damages in DNA of male mice spermatozoa. These resulted in ~26% decrease in fertility and gestation of female mice, along with an overall hormone secretion during the pregnancy, and ~39% reduction in viability of pups/pregnant females.

1. Introduction

Heavy quantum dots (QDs), *i.e.*, semiconductor nanocrystals (NCs) composed of heavy elements with small enough sizes to exhibit size-dependent characteristics, have received much attention because of their unique properties such as wide optical excitations, narrow, tunable and symmetric optical emissions, and also high photostability [1–4]. Furthermore, QDs (as highly efficient photo-absorbents providing multi-photon microscopy and imaging) [5] have been functionalized by biomolecules (such as DNA, proteins and/or antibodies) and/or coated by more biocompatible mineral layers (such as ZnS) [6] to provide selective targeting which is highly applicable in cell labeling, noninvasive tissue and cell imaging, *in vivo* tumor detection, photosensitization of photodynamic therapy, and drug delivery [7–11].

Among the heavy crystalline compositions, recently, CdTe (with a direct band gap of 1.44 eV at room temperature) has attracted many attentions, because CdTe QDs show tunable optical properties providing numerous applications in electronic areas (as it has been currently utilized in solar cells and light-emitting diodes). In addition, CdTe QDs have been potentially utilized for some *in vivo* cancer imaging [12,13].

Due to high demands for CdTe QDs, several different methods (such as chemical aerosol flow [14] and organic phase [15] synthesis methods) have been developed for synthesis of CdTe NCs with desired shapes and sizes. Although these kinds of methods can provide CdTe NCs with excellent quantum yield (QY), they are hydrophobic and incompatible with the aqueous biological media [16], and so, are not suitable for biological applications. So far, various methods have been suggested for synthesis of aqueous dispersible and more biocompatible CdTe QDs through surface modification of the QDs by silica [17], mercaptoacetic acid [18], micelles [19], liposomes [20], and amphiphilic polymers [21]. However, these synthesis methods not only are complicated and time consuming, but also the encapsulated QDs can be unstable (optically and/or chemically) in biological media.

* Corresponding author at: Department of Physics, Sharif University of Technology, P.O. Box 11155-9161, Tehran, Iran.
E-mail address: oakhavan@sharif.edu (O. Akhavan).

http://dx.doi.org/10.1016/j.msec.2016.06.055
0928-4931/

To overcome these problems, the aqueous phase synthesis technique was proposed as one of the best approach to produce large quantities of high quality CdTe QDs in a single-pot process [22–26]. The CdTe QDs synthesized through the aqueous phase technique generally show much higher QY (>50%) than the QDs synthesized firstly in organic solvents and then transferred to water (QY < 30%). In addition, surface of CdTe QDs synthesized by using the aqueous phase technique is terminated with reactive groups which provide directly conjugation of biomolecules to them for selective targeting and bioimaging applications.

All these advances indicate that there is a road map for application of the aqueous phase synthesized CdTe QDs (or generally, CdTe QDs exhibiting lower cytotoxicity) as one of the promising nanomaterials in biological and medical areas [27]. In addition, QDs have been proposed as serious competitors to complete or replace dye-sensitized solar cells (CdTe/CdS thin film cells have already captured ~10% of the global market), and have been utilized as components of sensors and emitting materials in LEDs [27]. Therefore, it is highly necessary for researchers and policy makers to understand all the probable toxic properties associated with heavy QDs. However, there is limited information about the health risks and toxicity of QDs. Several research groups are currently studying the toxicity of cadmium-based QDs for *in vitro* and *in vivo* cases [28–32]. Some studies indicated no detectable toxicity in small animals at optimum concentration of cadmium-based QDs for several months [33,34]. But, the fact that QDs possess heavy components, especially cadmium, raises serious doubts for using them in clinical trials [35]. Recently, Yong et al. [36] reported a concentration-dependent toxicity for CdTe QDs. They found no mortality by intravenously injection of CdTe QDs into SKH-1 mice at high concentration of 0.5 mg. However, by further increasing the concentration, >50% of the mice died, indicating the concentration is near the lethal dosage range. Although there are also some reports about isolation of CdTe QDs from the biological media with a capping such as ZnS [37], one cannot assure that all of the QD cores are completely covered with a smooth thickness of the shell, and so, some portions of the QDs are still susceptible for degradation and ion release. It should be also noted that the overall hydrodynamic size of the QDs should be minimized (<5–10 nm) to allow an effective renal clearance after performing their task in the body [38]. Furthermore, there are investigations which demonstrated that QDs (*e.g.*, $CdTe_xSe_{1-x}$/CdS QDs) are slowly removed from the body through hepatobiliary action. Therefore, the long term toxic effects of CdTe QDs remained in the body after a required injection should be further investigated. For example, the residual QDs can interfere in spermatogenesis and subsequently epigenesis, as previously reported for nanoparticles [39,40]. Furthermore, the epigenetic changes can negatively affect development of offspring.

In this research, at first, *in vitro* concentration-dependent toxicity of aqueous phase synthesized CdTe NCs on viability and motility of spermatozoa was studied. Using the fluorescence imaging, the uptake of the NCs by the sperm cells was monitored. Then, *in vivo* toxicity of the NCs on spermatozoa of male mice (viability and motility of the spermatozoa), fertility as well as gestation of female mice (female mice inseminated by the NC-treated male mice), and viability of pups/pregnant females were investigated.

2. Experimental section

2.1. Synthesis of CdTe NCs

CdTe NCs were synthesized through an aqueous-based solution method (green method) using NaHTe and $CdSO_4$ as the precursors and thioglycolic acid (TGA) as a capping agent (the chemicals were prepared from Merck). The method as previously reported elsewhere [26]. In summary, NaHTe solution was prepared by a sensitive reaction between sodium borohydride and tellurium powder. To do this, 7.3 mmol sodium borohydride and 7.0 mL deionized (DI) water were transferred to a flask. Then, 0.87 mmol tellurium powder (with Te/$NaBH_4$ molar ratio of 0.12) was added into the mixture of the flask and the mixture was vigorously stirred for about 3 h under purging of Ar gas flow. In this process the black tellurium powder was gradually disappeared and a white precipitate of sodium tetraborate was obtained at bottom of the flask. The clear NaHTe solution was rapidly transferred to 43 mL DI water which was pre-bubbled by Ar gas. In parallel, 3.92 mmol TGA was added into 150 mL $CdSO_4$ aqueous solution with 16.0 mM concentration. Then pH of the solution was adjusted ~9.5 using a few drops of 4 mM NaOH solution. After that, the freshly prepared NaHTe solution was injected into the Ar purged Cd-TGA solution and heated at 100 °C for 5.5 h. The molar ratio of Cd:Te:TGA was considered 2.75:1:4.5, in the final solution. For purification of the final solution, at first, a proper volume of acetone (with 1/10 volume ratio) was added into the prepared solution and centrifuged at 4000 rpm for 2 min (acetone results in temporary aggregation of the synthesized NCs). Then, the supernatant of the suspension (containing the residual ions) was discarded. This process repeated for several times (currently three times). The initial concentration of the CdTe in the centrifuged solution was ~10 mg/mL which determined through drying the solution at 60 °C in air and then weighing the remained powder. The other desired concentrations were obtained by diluting the initially high concentrated suspension with DI water. The finally prepared CdTe suspensions were sterilized by heating in an autoclave (already purged by Ar gas) at 120 °C for 15 min.

2.2. Material characterization

A Perkin-Elmer 55 luminescence spectrophotometer was utilized to obtain the photoluminescence (PL) spectra of the CdTe NCs. A Jasco V530 UV–visible spectrophotometer was used to determine the optical absorption property of the samples. The photoluminescence QY of the CdTe NCs was evaluated using the following relation: [41]

$$QY_{sample} = \left(\frac{F_{sample}}{F_{ref}}\right)\left(\frac{A_{ref}}{A_{sample}}\right)\left(\frac{n_{sample}}{n_{ref}}\right)^2 QY_{ref}$$

in which F, A, and n are integrated fluorescence intensity (obtained using area under the emission peak), absorbance at the excitation wavelength, and refractive index of the solvent, respectively. The QYs were determined relative to the QY of uranin ($C_{20}H_{10}Na_2O_5$) in water as a reference material. A Philips-CM200 transmission electron microscopy (TEM) apparatus operated at 200 kV was utilized to observe the CdTe nanoparticles. X-ray photoelectron spectroscopy (XPS) were done by using a hemispherical analyzer equipped by an Al Kα x-ray source ($h\nu = 1486.6$ eV) operating at a vacuum better than 10^{-7} Pa. The relative concentrations of the elements were calculated by using peak area ratio of the core levels and considering the sensitivity factor of each element in XPS. The XPS was also utilized to further study about uptake of the NCs by the spermatozoa. To do this, the NC-treated spermatozoa were rinsed by PBS (three times) to remove the NCs probably attached on surface of the cells. The spermatozoa were resuspended in PBS and centrifuged at 12,000 rpm for 30 s. Then, the obtained sperm pellet was dried at 120 °C in air for 1 h, loaded on a scotch tape, and transferred into a UHV chamber for XPS. The XPS data were calibrated by fixing the $Au(4f_{7/2})$ peak at 83.7 eV (a very thin Au layer was deposited on the samples by using a desktop sputtering system (Nanostructured Coating Co., Iran)). Zeta potentials of the NCs were estimated by using a Zetasizer (Malvern Instruments).

2.3. Animals

Male binaural alternate loudness balance (Balb)/C mice aged 8 weeks were maintained at 20 ± 2 °C on a 12:12 h light/dark cycle with *ad libitum* access to food and water. All animal procedures were performed based on the guideline of the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals.