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Cytotoxicity of MPA capped CdTe Quantum Dots on Saccharomyces cerevisiae

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Abstract

Water-soluble fluorescent CdTe quantum dots (QDs) were synthesized and non selective fluorescent labeling in yeast cells were studied with a fluorescence microscope.. Uptake of QDs by yeast cells became more prominent after 8 hours of incubation in 30 ^{0}C. Furthermore, cytotoxicity in yeast cells was studied both before and after exposing them to radiation. It was found that the radiation could be more destructive to a biological system in the presence of CdTe QDs than in its absence.

Keywords: Irradiation, Quantum Dots, Bio imaging, Yeast Cells, Cell Survivability.

1. Introduction

Luminescent semiconductor quantum dots (QDs) including II–IV and III–V semiconducting nanocrystals, have attracted worldwide research because of their excellent optical properties, such as size tunable fluorescence and narrow as well as symmetric emission profile with a broad excitation range [1-9]. These unique optical properties of QDs were exploited for applications, such as fluorescent markers in molecular and cellular labeling, imaging, sensing and diagnostics. In all such applications radiation directly interacts with QDs. Therefore, it is essential to know the way radiation interacts with such nanostructure and the changes it can impart to them. Furthermore, Ionizing radiation has also been employed for synthesis and modification in the properties of QDs [10-15]. Most of irradiation changes (such as size and shape) in QDs are due to restructuring or modification of surface of the quantum dots. For instance, after prolonged exposure of UV radiation on thioglycolic acid (TGA) capped water solvable CdTe QDs, a shell of CdS was formed on CdTe core [13] and similar studies using electrons was reported by the author with all details [12].

Semiconductor QDs are emerging as a new class of fluorescent labels for chemical analysis, cell imaging, and biomedical diagnostics [19-21]. Many researchers have used fluorescent water soluble CdTe QDs for such purposes. However, its application is limited due to presence of Cd^{2+} ion, which is toxic [22-28]. Furthermore, radiation as probe in bio-imaging can enhance release of cd $^{2+}$ ion which renders QDs as radiation sensitizing agent [29]. For in vivo and clinical imaging, the potential toxicity of QDs remains a major concern. Therefore, the study on cytotoxicity of CdTe QDs in presence and absence of radiation is useful In order to get insight of its practical applications in clinical imaging as well as radiation protector or sensitizer.

In the present study, colloidal MPA capped CdTe QDs were grown using hydrothermal method and irradiated by 8 MeV electrons. Various optical characterization techniques such as steady state and time resolved photo luminescence (PL) and UV-Visible absorption spectroscopy were used to find the changes before and after irradiation on QDs properties. In order to understand cytotoxicity of QDs, wild type yeast strain *Saccharomyces cerevisiae* X2180 was treated with MPA capped CdTe QDs and effect of concentration of CdTe QDs on cell viability was studied. In addition, cell cytotoxicity was measured in presence and absence of radiation.

2. Experimental Details

2.1Materials and methods

Na2TeO3 and CdCl2 as precursors and Mercapto Propionic Acid (MPA) as surface stabilizer are procured from Sigma Aldrich and the yeast cell type *Saccharomyces cerevisiae* X2180 was acquired from BARC, Mumbai, India.

2.2. Preparation of MPA capped CdTe QDs

A simple efficient hydrothermal method was used for the synthesis of Mercapto Propionic Acid (MPA) capped CdTe QDs. Trisodium Citrate Dehydrate (100 mg), Sodium Tellurite (0.01 mol/L, 4 mL), and Sodium Borohydride (50 mg) were successively added to 4 mL of Cadmium Chloride solution ($CdCl_2$, 0.04 mol/L) and diluted by adding 32 mL ultra-pure water. To the prepared solution, MPA was added to obtain the growth solution for MPA capped CdTe QDs. The solution was autoclaved in stainless steel autoclave with Teflon liner at 180 °C for 45 min. then,the grown QDs were washed twice with IPA to remove unwanted Cadmium and Tellurium ions and stored at 4 ^{0}C in dark place.

CdTe colloidal QDs were irradiated with electron doses ranging from 0.2 kGy – 2.6 kGy using 8 MeV Microtron Accelerator available at Mangalore University, India. The samples were taken in microtubes and exposed to 8 MeV electrons at a distance of 30 cm from the beam exit port of the Microtron accelerator. The details of the facility are reported elsewhere [30]. Samples were characterised for changes before and after irradiation. Absorption spectra were recorded using Shimadzu UV - 3101PC double beam spectrophotometer. TCSPC studies were carried out using Chronos BH and Edinburgh FLS 920. Picoseconds LEDs (excitation wavelength of 320 nm – 450 nm, pulse width 750 ps and 1MHz rep rate) were used as excitation source in the present study.

2.3. Yeast Samples preparation

A wild type diploid yeast strain *Saccharomyces cerevisiae* X2180 was used for the study. The single cell stationary-phase cultures were obtained by growing the cells on Yeast extract: Peptone: Dextrose (YEPD) (1%:2%:2%) medium for several generations in exponential phase to a density of approximately 30,000 cells mL^{-1}. Cells were washed thrice by centrifugation (1,500 RPM for 10 min) and re-suspended in DI water. Cell concentration was kept around 30,000 cells mL^{-1} (by counting in haemocytometer) in a sterile polypropylene centrifuge tube for irradiation and cytotoxic study.

2.4. Survival assay

In order to study survival assay, yeast cells were suitably diluted and plated in triplicate on YEPD agar medium. Plates were incubated for 48 h at 30 ^{0}C in the dark, and the outgrowing colonies were counted. The data points in all figures in the results are average of three independent experiments. Error bars in all figures indicate the standard error of the mean.

3. Results and Discussion

3.2.1. Non-selective labelling of yeast cells

Cell labelling is one of the important applications of CdTe QDs**.** Before carrying out radiation sensitization studies of CdTe QDs, QDs uptake was monitored using non selective cell labelling.

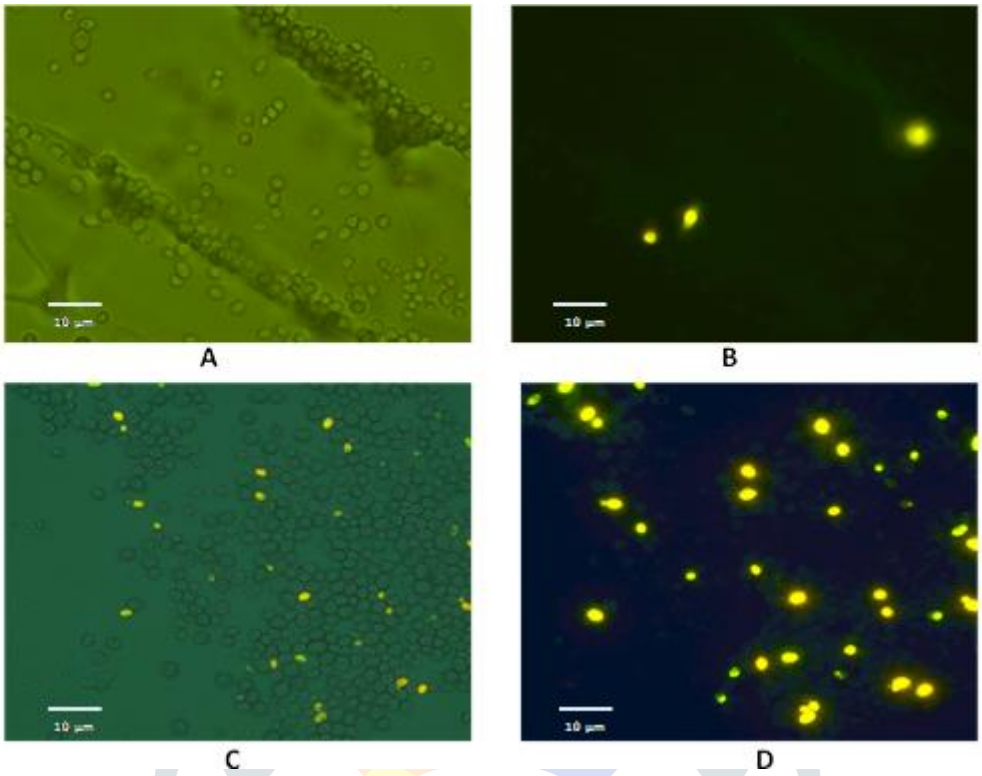

Figure 6. Labelling of living yeast cells using QDs. (A) Bright field and (B) fluorescent images of cells taken after 4 h incubation with orange QDs. (C) Bright field and (D) fluorescent images of cells taken after 8 h incubation with orange QDs

The uptake (endocytosis) of MPA capped CdTe QDs by *Saccharomyces cerevisiae* X2180 yeast cells was studied using fluorescence optical microscopy. Wild-type yeast cells were grown in YEPD medium and collected by centrifugation. The cells were cooled on ice, and were mixed with orange color QDs of concentration 50 nM. After 15 min on ice, the sample was warmed to room temperature and incubated in water bath shaker at 30 ^{0}C. Its uptake was monitored under fluorescence microscope. A bright field and fluorescent images of same sample were taken after 4 hours and 8 hours of incubation. It can be seen from figure 6 that the uptake was apparent after 4 hours incubation at 30^{0}C.

3.2.2. Concentration dependent Yeast cell viability.

Electron irradiation on CdTe QDs can cause photo oxidation and photo corrosion of CdTe QDs surface. This may lead to release of free Cd^{2+} ion [25]. Since, radiation is a probe in bio labeling, can alter the viability of cell by enhancing release of Cd^{2+} ions [29] [43]. Therefore, a study on effect of radiation on survivability of labeled cell can be very useful.

CdTe QDs can release cadmium into solution; even small amounts of leached cadmium can have significant effects on cell survivability [44, 45] as this trace metal is acutely toxic. It is well known that, cell survivability is strongly dependent

on concentration of QDs Therefore, it is very essential to know lethal and sub lethal concentration of the quantum dot on cell survivability.

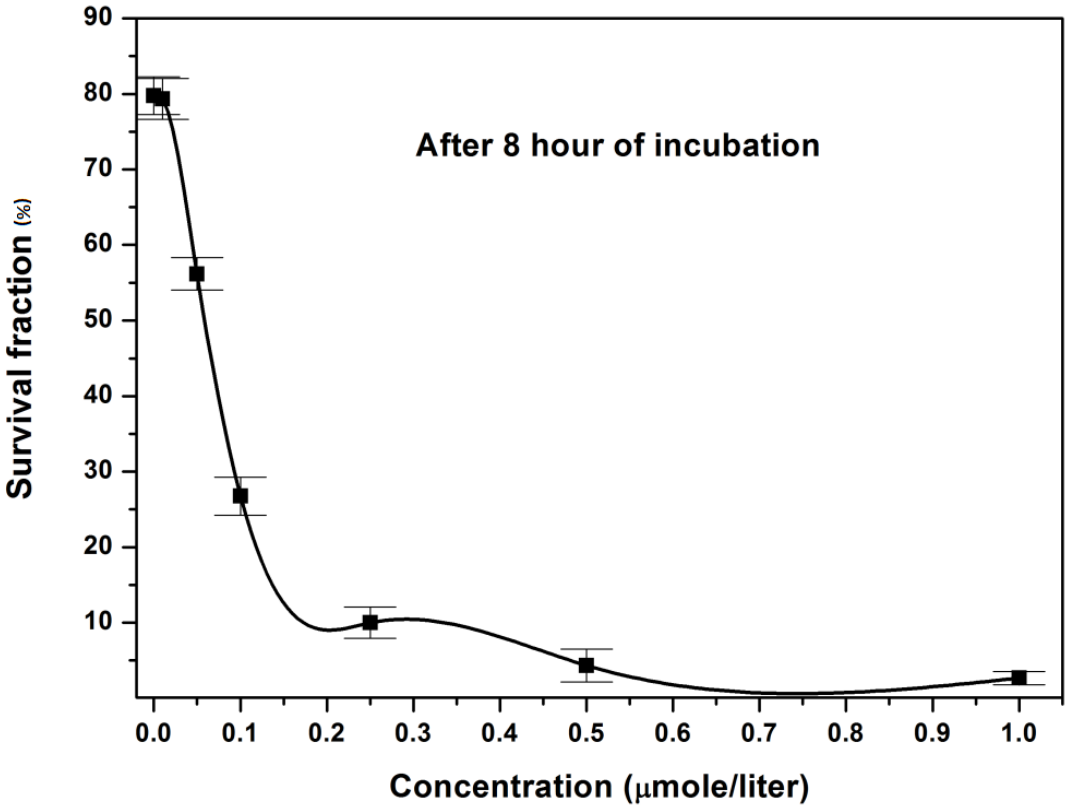

Figure 7. Survival fraction of Yeast cell for different concentration of CdTe QDs

The cell viability study of MPA capped CdTe QDs on yeast cell was carried out after 8 hour of incubation. CdTe QDs were incubated with yeast cell in water bath shaker (2 Hz) at 30 ^{0}C. Concentration of CdTe QDs was varied from 10 nM to 1μM, keeping yeast cell concentration constant, and survival fractions were measured for each concentration. Survival fraction as a function of concentration of CdTe QDs was plotted and is shown in figure 7. Cell viability at concentration of 10 nM is not significantly altered as compared to that of the control, untreated cells. At medium concentrations of CdTe QDs (50nM to 250 nM), although the number of viable cells was decreased, saturation in cell viability observed only after 250 nM. A high dose (250 nM to 1μM) treatment of CdTe QDs completely inhibited cell growth. This inhibition is due to combined effect of intracellular release of Cd^{2+} ions [46] and other free radicals generated by CdTe QDs [47, 48].

3.3.3. Survival response of QDs sensitized yeast cells to irradiation.

Electron irradiation is known to cause damage in living cells and tissues. Addition of radiation sensitizing agent can accelerate the damage of cells. CdTe QDs consist of cadmium which is cytotoxic can enhance radiation damage. Therefore, radiation damage studies of yeast cell treated with QDs were carried out to ascertain enhanced damage in living cells with presence of QDs.

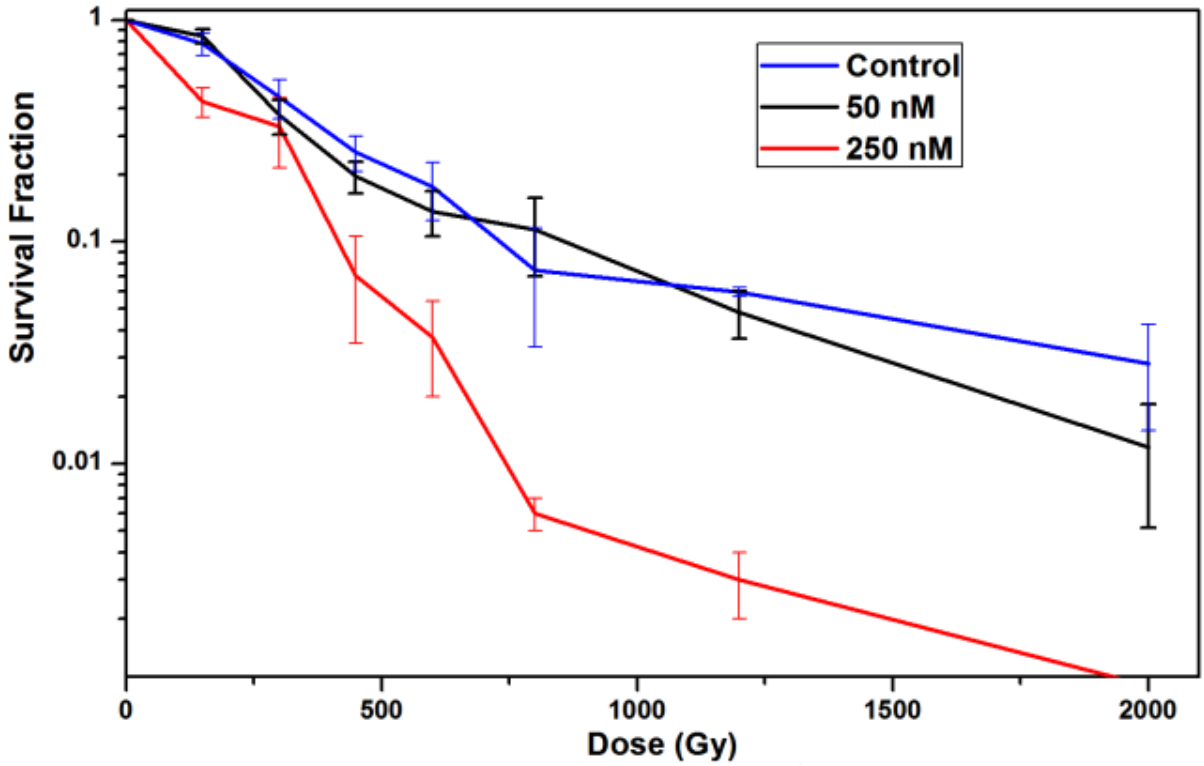

Figure 8. Plot of log of survival fraction for different radiation doses.

In order to understand cytoxicity of QDs in presence of radiation, yeast cells were treated with of CdTe QDs of concentration of 50 nM and 250 nM. Where 50 nM and 250 nM correspond to lethal and sub lethal concentration of QDs respectively. Yeast cells without QDs treatment were taken as control. Yeast cells were incubated for 8 hours before irradiation to ensure complete uptake of quantum dots. Thus treated cells were exposed to radiation under euoxic conditions and the survival fraction was found out. The dose response of yeast cells, a plot of log of survival fraction with respect to dose in Gys was plotted and is shown in Fig. 8. These curves were fitted to multi target model $S = 1 - (1 - e^{-KD})^{n}$ where S is survival rate of yeast cells, D is absorbed dose (Gy), K is inactivation constant and n is extrapolation number. Observed parameter values from the theoretical fit are shown in table II. A good fit ($R^2 > 0.95$) of multi target model to sigmoid curve was observed due to the multi hit processes combined with molecular repair processes survival. From the least square fit values, the survival response for *Saccharomyces cerevisiae* X2180 under euoxic condition can be represented as $S = 1 - (1 - e^{-0.00413D})^{1.83}$ for control, $S = 1 - (1 - e^{-0.00437D})^{1.88}$ for 50 nM treated sample and $S = 1 - (1 - e^{-0.0071D})^{1.86}$ for 250 nM treated sample. The reciprocal of inactivation constant (1/K Gy) for control, 50 nM and 250 nM were 242 Gy, 228Gy and 140Gy respectively. Decrease in inactivation constant and narrow shoulder at sublethal dose was observed with increase in concentration of QDs. This may be due to the increased rate of production of free radicals such as Cd^{2+} produced by electron beam and subsequent breaking of DNA strand [25] [29] [49] Therefore a dose of radiation can be more destructive to a biological system in the presence of CdTe QDs than in its absence.

Table 2 : Multitarget fit parameters for different concentartion and radiation doses.

Parameters	Control (without quantum dot)	Quantum dot concentration 50nM	Quantum dot concentration 250nM
K	0.00413±1.3E-4	0.00437±3.6E-4	0.0071±6.29E-4
N	1.83±0.05	1.88±0.03	1.86±0.08
D_0	242.2±0.04	228±0.08	140±0.08

4. CONCLUSIONS

. Fluorescent labeling and radiation sensitization effect of CdTe QDs in yeast cells were also performed. QDs endocytosis became more prominent after 8 hours of incubation at 30 ^{O}C which was clearly observed from fluorescence images of yeast cells taken by florescence microscope. Cytotoxicity study shows that QDs concentration more than 250 nM is lethal to cell survivability. Yeast cell became more sensitive to irradiation after treating them with QDs.

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