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Rhizopus stolonifer mediated biosynthesis of biocompatible cadmium chalcogenide quantum dots

P. Mareeswari [a], J. Brijitta [b,*], S. Harikrishna Etti [c], C. Meganathan [c], Gobi Saravanan Kaliaraj [b]

[a] Department of Electronics and Communication Engineering, G.K.M. College of Engineering and Technology, Chennai 600 063, Tamil Nadu, India
[b] Centre for Nanoscience and Nanotechnology, Sathyabama University, Chennai 600 119, Tamil Nadu, India
[c] Department of Physics, G.K.M. College of Engineering and Technology, Chennai 600 063, Tamil Nadu, India

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ABSTRACT

We report an efficient method to biosynthesize biocompatible cadmium telluride and cadmium sulphide quantum dots from the fungus *Rhizopus stolonifer*. The suspension of the quantum dots exhibited purple and greenish-blue luminescence respectively upon UV light illumination. Photoluminescence spectroscopy, X-ray diffraction, and transmission electron microscopy confirms the formation of the quantum dots. From the photoluminescence spectrum the emission maxima is found to be 424 and 476 nm respectively. The X-ray diffraction of the quantum dots matches with results reported in literature. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay for cell viability evaluation carried out on 3-days transfer, inoculum 3×10^5 cells, embryonic fibroblast cells lines shows that more than 80% of the cells are viable even after 48 h, indicating the biocompatible nature of the quantum dots. A good contrast in imaging has been obtained upon incorporating the quantum dots in human breast adenocarcinoma Michigan Cancer Foundation-7 cell lines.

1. Introduction

There has been a great bloom in the green synthesis of nanoparticles, as it limits the usage of toxic chemicals used in the chemical methods of synthesis. It has been well documented in the literature that both unicellular and multicellular microbial organisms and plant extracts can biosynthesize nanoparticles [1–8]. The biosynthesis can be either intra-cellular or extra-cellular resulting in particles whose size, shape and chemical composition can be controlled. Though, the yield from bio-synthesis is low as compared to established chemical routes, it offers biocompatibility and an eco-friendly approach and holds all the physico-chemical properties of the chemically synthesized nanoparticles. Semiconductor quantum dots (QDs) are one among the several bio-synthesized nanoparticles. They possess unique properties such as size tunable emission colour and narrow emission profile [9–12] and are free of photobleaching [13–15] and these properties can be effectively tapped for imaging applications [16–20]. Numerous literature reports are available pertaining to the biosynthesis of QDs from microbial organisms such as *Fusarium oxysporium* [21], *Schizosaccharomyces pombe* [22], *actinomycetes* [23], *Rhodopseudomonas palustris* [24], *Phanerochaete chrysosporium* [25], *Rhodobacter sphaeroides* [26], *Gluconoacetobacter xylinus* [27], *Escherichia coli* [28], etc.

Bao and co-workers [29,30] reported the extracellular biosynthesis of cadmium telluride (CdTe) QDs from *Escherichia coli* and *yeast* cells. They reported that a surface protein capping layer improves the biocompatibility of the CdTe QDs. Syed and co-workers [31] investigated the anti-bacterial activity of biosynthesized CdTe QDs by the fungus *Fusarium oxysporum* and found that the QDs inhibit the growth of both gram positive and gram negative bacteria. Sweeney and co-workers [32] proved that the genetic and physiological parameter enhances the formation of cadmium sulphide (CdS) nanoparticles within bacterial cells. Holmes and co-workers [33] understood from atomic absorption spectroscopy, inductively coupled plasma mass spectrometry, and acid-labile sulfide analysis that CdS nanoparticles in the range of 20–200 nm are formed extracellularly in *Klebsiella aerogenes*.

In this paper we report the biosynthesis of cadmium chalcogenide compounds CdTe and CdS QDs using the fungus *Rhizopus stolonifer* which is commonly known as the black bread mold. To the best of our knowledge, this is the first report on the biosynthesis of CdTe and CdS QDs from *Rhizopus stolonifer*. The quantum dots

* Corresponding author.
E-mail address: brijitta@live.com (J. Brijitta).

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are found to form extracellularly and exhibits good crystallinity as evident from X-ray diffraction studies. The photoluminescence spectra of the suspension of the QDs confirm that, the observed luminescence falls in the visible region of the spectrum. Studies shows that the QDs are biocompatible and it is possible to image with good contrast the Michigan Cancer Foundation-7(MCF-7) cell lines loaded with QDs.

2. Experimental

2.1. Materials and methods

Cadmium perchlorate hexahydrate ($Cd(ClO_4)_2{\cdot}6H_2O$), tellurium tetrachloride ($TeCl_4$), Zinc sulphide (ZnS) and potato dextrose agar (PDA) are purchased from Sigma-Aldrich. Dust free ultrapure water (Millipore, 18.2 MΩ) is used throughout the biosynthesis. All the chemicals used for the synthesis are of analytical grade. *Rhizopus stolonifer* is procured from Institute of Microbial Technology, Chandigarh, India and is maintained on PDA at 25 °*C* for 15 days. From the stock culture, sub-cultures of *Rhizopus stolonifer* are inoculated in 500 mL of PDA broth and incubated at 25 °C for 7–10 days. For the studies, the fungal mycelial mass is separated from the culture broth by filtration and washed several times with ultrapure water.

2.2. Biosynthesis of quantum dots

For the biosynthesis of CdTe QDs, 20 g of the wet mycelial mass is dispersed in an Erlenmeyer flask containing 100 mL of an aqueous solution of 1 mM $CdCl_2$ and 1 mM $TeCl_4$. The solution is kept in a shaker at 200 rpm and the temperature is maintained at 25 °C for a 96 h. Appearance of purple luminescence in the solution upon illuminating with an 8 W UV lamp indicates the formation of CdTe QDs in the solution. The biosynthesis of CdS QDs is carried out by dispersing the mycelia in 100 mL aqueous solution of 1 mM Cadmium chloride and 1 mM ZnS. Though ZnS is water insoluble, upon the addition of the mycelial mass, ZnS is found to dissolve. *Rhizopus stolonifer* is known to produce acids such as, fumaric acid, lactic acid [34], these acids could have aided in the solubility of ZnS in water. The CdS QDs is synthesized by adopting the same methodology for CdTe QDs. A greenish-blue luminescence is observed upon UV light illumination; this indicates the formation of CdS QDs. The fungal biomass is removed from both the QDs solution by filtering using a 0.22 μm membrane filter.

2.3. Characterization

X-ray diffraction (XRD) patterns have been recorded using a Rigaku, Smartlab, (Japan) diffractometer using Cu Kα radiation of wavelength $\lambda = 0.1541$ nm in the scan range of $2\theta = 20 - 75^{\circ}$. Transmission electron microscopy (TEM) images were taken with a FEI-TECHNAI, G2-MODEL (T-30 STWIN) operated at an acceleration voltage of 250 kV. Samples for TEM analysis were prepared by dropping the QDs suspension on the copper grids followed by drying the grids at room temperature. Room temperature photoluminescence (PL) measurements were carried out using a He-Cd laser (325 nm) as the excitation source with backscattering geometry. The laser beam impinging on the QDs suspension is tuned to 25 mW. The backscattered light from the QDs suspension is dispersed by a triple grating spectrometer, Jobin Yvon-Horiba T64000 system. Liquid nitrogen cooled CCD has been used to record the PL spectrum.

2.4. MTT assay

The MTT assay is a well-established nonradioactive colorimetric assay to measure the cell cytotoxicity, proliferation, or relative cell viability. In this assay the yellowish tetrazolium MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) dye is reduced by metabolically active cells present in the mitochondria to form the insoluble purple coloured formazan crystal. The formazan crystal is dissolved in dimethylsulphoxide (DMSO) solvent and is quantified by recording the spectroscopic absorbance values. The procedure adopted for MTT assay in this work is described below.

The NIH 3T3 embryonic fibroblast cells from albino Swiss mouse are plated separately in 96 well plates at a concentration of 1×10^5 cells/well. After 24 h, the cells are washed twice with 100 μL of serum-free medium and starved for an hour at 37 °C. After starvation, cells are treated with the CdS and CdTe QDs for 24 h. At the end of the treatment period the medium is subjected to aspiration and serum free medium containing MTT (0.5 mg/ml) is added and incubated for 48 h at 37 °C in a CO_2 incubator. The MTT containing medium is discarded and the 3T3 cells are washed with 200 μL phosphate buffer solution. The formazan crystals are dissolved by adding 100 μL of DMSO and pipetted it up and down for uniform mixing. Spectrophotometrical absorbance of the purple blue formazan dye is measured using a Biorad, Model-680 microplate reader at 570 nm. The percentage cell viability is calculated from the following equation,

$$Cell \quad Viability \quad (\%) \quad = \quad \frac{I_{Cells+QDs}}{I_{Cells}} \times 100$$

where $I_{Cells+QDs}$ is the fluorescence intensity of the 3T3 cells incorporated with the QDs and I_{Cells} is the fluorescence intensity of 3T3 cells with the culture medium only.

2.5. Imaging QDs loaded in cancer cell line

Localization of samples has been carried out in human breast adenocarcinoma cell line (MCF-7). Briefly, 2×10^4 cells/well of MCF-7 cancer cell line is seeded on 96-well Cell Carrier microplates. After attaining 80% confluence the media is changed and then the cells are treated with CdTe and CdS QDs and incubated the cells for 24 h in a humidified incubator at 37 °C with 5% CO_2. The cells are washed twice with ice cooled phosphate buffer solution. The localization of the QDs in the live cells is imaged using a Nikon Eclipse Ti-E Inverted Microscope.

3. Results and discussions

Fig. 1 shows the photographs of the suspension of CdTe and CdS QDs under normal and UV light illumination. The suspension of CdTe and CdS QDs show purple (Fig. 1c) and greenish-blue (Fig. 1d) luminescence respectively upon illuminating using an 8 W UV lamp (Philips Lighting, TL, Poland, peak wavelength 370 nm).The suspensions of both the QDs are extremely stable and did not show aggregation even after four months of storage.

The X-ray diffraction pattern of theCdTe and CdS QDs is shown in Fig. 2a and b. For both the CdTe and CdS QDs the XRD pattern exhibits broad peak with well refined pattern. Line broadening of the XRD peaks indicates that the size of the CdTe and CdS QDs fall in the domain of nano range. The strong diffraction peaks indicates that the CdTe and CdS QDs possess good crystallinity. By applying the Debye-Scherrer formula, the average crystallite size of the QDs can be estimated from the full width at half-maximum (FWHM) of the diffraction peaks. The Debye-Scherrer formula is given by,

$$d \quad = \quad K \times \lambda / B \times Cos\theta_B \qquad (1)$$