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ARTICLE

Crucial factors in biosynthesis of fluorescent CdSe quantum dots in *Saccharomyces cerevisiae*

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Cadmium selenium (CdSe) quantum dots (QDs) were synthesized using *Saccharomyces cerevisiae* (ATCC9763) as a biomatrix cultured with 5 mmol/L Na₂SeO₃ and 1 mmol/L CdCl₂. Five decisive factors in the biomanufacture of CdSe QDs in *S. cerevisiae* were investigated and optimized, including the time point of adding Na₂SeO₃, the optimal concentrations of selenite and cadmium, and the incubating duration of selenite and cadmium with yeast cells respectively. By exploiting two dependent metabolic pathways in an appropriate procedure, CdSe QDs were obtained simply with controllable particle size and tunable fluorescence emission. With the help of an effective extracting method, the maximum emission peaks of different isolated QDs had been further detected, with adjustable wavelengths ranged from 506 nm (green) to 562 nm (yellow). The following in vitro 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay revealed that the biocomposed CdSe QDs had much lower cytotoxicity compared with hydrothermally synthesized thioglycolic acid (TGA)-capped CdSe and TGA capped-CdTe. By the advantages of the operational protocol, these findings exhibited the potential of an uncomplicated biosynthetic method which is capable of producing easily detectable, environment-friendly and low toxic inorganic nanoparticles with easy access of raw materials.

Keywords: *Saccharomyces cerevisiae*; Yeast; QDs; bio-matrix; MTT; nanocrystals.

Introduction

Quantum dots (QDs), also known as semiconductor nanocrystals, have unique physical and optical properties^{1, 2}, which lead to an extensive application in the fields of biosensing^{3, 4}, imaging⁵ and solar cells^{6, 7}. Traditionally, QDs were produced by precursor thermal decomposition, hot-injection method and hydrothermal method^{5, 8, 9}. However, all of the routes mentioned for the QDs synthesis involve environment-unfriendly procedures. Some reactions for the QDs' generation are under anaerobic, anhydrous and high-temperature conditions. While some were carried out in an airtight container with high pressure for manufacture of high-

quality QDs. Furthermore, some reagents in chemical reactions are toxic, flammable, and even explosive.

Recently, biosynthesis methods by using biological matrices to synthesize QDs has attracted broad attentions. It offers an alternative route to achieve hydrophilic and biocompatible QDs which also prove to be controllable in size, shape and even in function decoration^{10, 11} under the ambient temperature and atmospheric conditions. Different biological matrices, such as bacteria *Escherichia coli*^{12, 13}, photosynthetic bacteria *Rhodospseudomonas palustris*¹⁴, fungus *Schizosaccharomyces pombe*¹⁵ and *Fusarium oxysporum*^{15, 16} had already been used for the biomanufacture of cadmium sulphide (CdS) QDs in responses to cadmium salt. Also cadmium telluride (CdTe)¹⁷⁻¹⁹ and cadmium selenide (CdSe)^{3, 20} were produced by bacteria or fungi for their anticipated wide applications. Biosynthesis technique as a novel green protocol is not only low-cost and environment friendly, but the metabolic pathway of the organism can also endow the newly formed nanocrystals with the advantages of highly hydrophilic and bioavailable^{20, 21}. However, some essential components of biosynthesis in bacteria include the optimal working concentration of reagents, the suitable growth stages for introduction of biosynthetically ingredient and the incubating duration with bio-matrices, which in all determine fluorescent quantum yield and optical properties of QDs, have not been investigated systematically yet.

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In the work, CdSe QDs were selected as the target QDs due to its easy access of raw materials and wide utilities in biology. In the biosynthesis, *Saccharomyces cerevisiae* (*S. cerevisiae*) as a matrix was used to incubate with Na_2SeO_3 and CdCl_2 sequentially and respectively with appropriate concentrations at pre-designed time points. Researches^{20, 22} have shown that biosynthesis of QDs depends on the bacterial metabolic processes especially on the intracellular detoxification activities, which are able to be triggered by selenite²³ or other sub-lethal concentrations of toxic heavy metals^{24, 25}. Consequently, the abilities of bacterial heavy metals' defenses relied heavily on their physiological status, dose of toxic reagents and the time of toxin exposure. Hence, the five key points of QDs biosynthesis, including growth phase of bacteria, incubating concentration of Na_2SeO_3 , incubating duration with bacteria after the addition of Na_2SeO_3 , concentration of CdCl_2 and its incubating duration with the matrices, demand systematically investigation and optimization. Further on, these biogenic QDs were isolated to closer detect their fluorescence intensities, size distribution as well as cell cytotoxicity by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, which utilized hydrothermally synthesized thioglycolic acid (TGA)-capped CdSe and TGA capped-CdTe as controls. The aim of the work is to study the effects of the five crucial factors on the biomanufacture of CdSe QDs in *S. cerevisiae*, displaying potentials of the biosynthetic technique which provides to manufacture easy detectable, environment-friendly and low toxic inorganic nanoparticles with easy access of raw materials and handy process.

Materials and Methods

Biosynthesis and characterization of CdSe QDs

Five important factors of biosynthesizing QDs have been investigated in order, as showed in figure 1. The biosynthetic schemes were as follows: *Saccharomyces cerevisiae* (ATCC9763) was cultured in a sterile test tube (18 mm×180 mm) with 5 mL yeast extracted peptone dextrose (YPD) medium for 18 h, at 250 rpm/min, 30 °C. Then 200 μL of this preculture was inoculated into 100 mL YPD, cultured at the same conditions for 16 h, 20 h, 24 h and 28 h individually (factor 1: the time point of adding Na_2SeO_3). After that, 10 mL of culture above was transferred into a 50 mL sterilized flask respectively, adding 0, 1, 3, 4, 5 and 8 mmol/L Na_2SeO_3 separately (factor 2: Na_2SeO_3 concentration) and cultivated for 0, 12, 14, 16, 18, 20, 22, 24 and 26 h individually (factor 3: Na_2SeO_3 incubating duration). Yeast pellets from 10 mL culture medium were harvested by centrifugation at 4,000 rpm for 4 min, then resuspended in 10 mL of YPD containing 0.5, 1, 2, 3, 5 and 7 mmol/L CdCl_2 solution respectively (factor 4: cadmium concentrations). These mixtures were finally cultivated in the dark for 14, 26 and 44 h (factor 5: CdCl_2 incubating duration) to obtain desired CdSe QDs with different colors of fluorescence.

The suspensions of yeast cells were obtained by centrifugation at 7000 rpm for 3 min, washed for three times and resuspended with 0.01 mol/L phosphate buffer solution (PBS, pH 7.4). Yeast cells were then adjusted to the same optical density OD_{560} (in a same batch) by UV-Vis spectrophotometer before observation. These 5 factors were performed one by one. When one variable was being researched, the variables which were not being investigated are similar to the published work²². Other wise, the best performing variability of a factor from the preceding stage was taken up in the following stage.

Alternative synthesis method, named as "One Pot" method was also carried out. Na_2SeO_3 and CdCl_2 were introduced into broth simultaneously when bacteria were cultured to the same growth phase. And then, the mixture was co-incubated for 24 h before harvesting.

Characterization of CdSe QDs

The optimal biosynthesis conditions were quickly assessed according to fluorescence intensity of harvested yeast cells treated under different procedures. The fluorescence excitation spectra of all samples were measured with a fluorescence spectrophotometer (RF-5301, Shimadzu; $\text{Ex}=335\text{nm}$) using the same settings. Images of cells were collected using a fluorescence inverted microscope Olympus IX71 (Tokyo, Japan) and a laser scanning confocal microscope Olympus IX81 (equipped with FV10 ASW2.1 software, $\lambda_{\text{ex}}=405\text{nm}$).

Isolation of CdSe QDs from bacteria

Bacteria containing CdSe QDs were pretreated with 0.3% sodium thioglycolate for 30 min, followed by incubated with glucuronidase at 42°C for 12 h in isotonic sorbitol solution (5.48%). Then the yeast cells were harvested by centrifugation at 4,000 rpm for 4 min and resuspended in PBS (0.01M, pH 7.4) and then subjected to ultrasonic treatment (JY92-II, Scientz Biotechnology Co., Ltd., China) for 5s and repeated for 25 times with intermittent of 10 s. The treated organisms were crushed with acid-washed glass beads (425–600 μm , Sigma, USA) for 1 h on a vortex. The suspension was followed by centrifugation at 10000 rpm for 15 min to remove the bacterial fragments. Then small molecules and ions (the molecular weight less than 100 kDa) were also removed by ultrafiltration tubes (Amicon Ultra-4,100k, Millipore Corporation, USA).

The transmission electron microscopy (TEM) was then used to check the size distribution of isolated QDs (Hitachi H-7650 with an acceleration voltage of 80 kV and a magnification of 30.0k). The absorption spectra of the isolated CdSe QDs were measured with a UV-1800 UV-Visible spectrophotometer (Shimadzu, Japan). Energy dispersive spectroscopy (EDS) was taken with a JEOL JEM-2010F instrument operated at an accelerating voltage of 200 kV.

Cytotoxicity tests and cell morphology observation

MTT assay, a standard cell viability assay, was taken to test cytotoxicity of the QDs according to the published protocols^{26, 27}. Human breast adenocarcinoma cells (MCF-7 cells) were

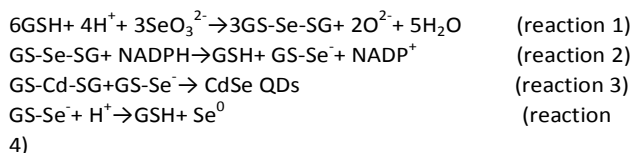
used as model cells. Hydrothermally synthesized CdSe²⁸ and thioglycolic acid (TGA)-capped CdTe²⁹ QDs were chosen as controls. All three kinds of QDs were sterilized by filtration tubes (equipped with 0.2 μm membranes), diluted in medium to different final concentrations (0, 100, 200, 500, 1000 and 2000 nM) and cultured with cells for 48 h. Then, each well was washed three times with fresh medium to remove excess QDs. All measurements were performed triplicately.

The rat INS-1 cell line, from the American Type Culture Collection (Manassas, VA, USA) was used for cell morphology observation. Biosynthesized CdSe QDs and two kinds of hydrothermally synthesized QDs were incubated with INS-1 cells for 48 h at the same concentrations as positive controls. Fluorescent micrographs were achieved by Olympus IX71 inverted fluorescence microscope equipped with a 40x oil-immersion lens.

Theory

Based on the researches published^{22, 30-32}, the mechanisms of CdSe biosynthesis are complicate and can be summarized as reactions 1-3. First of all, glutathione (GSH) is prone to react with intracellular Na₂SeO₃ to produce selenosulfide derivatives such as selenodiglutathione (GSSeSG) (reaction 1)³⁰ and selenoglutathione (GSSeH) (reaction 2), which is one of the important selenium precursors for CdSe formation (reaction 3). Secondly, GSSeSG, as a substrate for GSH-related enzymes such as thioredoxin reductase and glutathione reductase^{31, 32}, is able to be reduced into kinds of low-valence organoselenium, such as selenocystine [(Cys-Se)₂] and selenomethionine (Se-Met) in yeast cells, which have been verified by HPLC-ICP-MS²² and also considered as selenium precursors in CdSe synthesis.²² When CdCl₂ is subsequently added, some of them will be transported into the bacterial cells and lead to rapid and spontaneous formation of bis(glutathionato)cadmium (Cd•GS₂).³³ Then, the precursor GSSe⁻ could react with Cd•GS₂ and produce CdSe by accurate designation of experimental procedure (reaction 3).

Researches also reveal that some ATP-energized transporter conferred resistance to cadmium salts may transport this Cd•GS₂ into vacuole for decreasing the metal toxicity to a minimum.³⁴ Hence, starting incubating bacteria with CdCl₂ to get Cd•GS₂ at the right moment is necessary to make sure the reduced selenium and cadmium meet outside the vacuole for CdSe QDs synthesis.



Kessi, J. et al³⁰ has reported that there is another route of selenite detoxification (reaction 4), which will cause the failure of QDs synthesis or at least decrease the synthetic efficiency as well. GS•Se⁻ is unstable and easily reduces into red elemental Se (Se⁰) causing the accumulation of Se particles in cytoplasm or in the culture medium (reaction 4). The reaction has been

studied in some kinds of microorganisms such as *Escherichia coli*³⁰, *Bacillus subtilis*³⁰ and *Rhodospirillum rubrum*³⁵. Because the oxidation state of selenium, SeO₃²⁻ and SeO₄²⁻ (selenite and selenate), are highly toxic and soluble, thus a number of microorganisms tend to finally reduce them into elemental selenium (Se⁰, in short), which is a much less toxic and insoluble form.³⁶ As a result, the color of culture broth will turn from orange into red, which is the color of elemental Se⁰ (SI figure1, SI: supporting information). Apparently, it will decrease the concentration of potential selenium precursor for CdSe QDs biosynthesizing. Therefore, decreasing the formation of Se⁰ and maximizing the Se precursor is also required and much effective in increasing CdSe production because only organoselenium instead of Se⁰ can be used for the biosynthesis.

Results and discussion

1. Five key factors affected on the biosynthesis of QDs

1.1 The time point of adding Na₂SeO₃: effect of *S. Cerevisiae*'s growth phase

The efficiency of SeO₃²⁻ reduction and Se precursor's formation by bacteria varied in different growth phases of yeast *S. cerevisiae*.²² Therefore, it was necessary to choose a suitable time to add Na₂SeO₃ when yeast was physically ready to reduce it with high productivity. Growth curve of *S. cerevisiae* was firstly plotted (SI figure 2), showing at least three phases of growth: lag phase (0-10 h), log phase (11h-27 h) and stationary phase (28h-32h). Since bacteria were not physically prepared and had a small number in lag phase, bacterial log phase which could be further divided into early-log phase, mid-log phase and late-log phase attract considerable attentions.

In experiments, Na₂SeO₃ was mixed and dissolved with broth media when yeast cells grew to early-log phase, mid-log phase, late-log phase and stationary phase (16 h, 20 h, 24 h and 28 h) respectively to observe the yields of QDs which were measured by the maximum intensities of fluorescence peaks of harvested cells. As shown in figure 2a and 2b, the maximum intensity of fluorescence peaks increased with the time of adding Na₂SeO₃ extending from early logarithmic phase of bacteria to stationary phase one's (from 16 h to 28 h exactly). However, the fluorescence of QDs could not be observed obviously except slightly cellular auto-fluorescence when the same concentrations of Na₂SeO₃ were introduced in the rest growth phases of *S. cerevisiae* (data not shown).

And the maximum fluorescent intensity emerged when Na₂SeO₃ was input at the stationary phase yeast as a bio-matrix. It was mainly because that *S. cerevisiae* could synthesize GSH and related reductive enzymes more effectively in stationary phase²⁰ when Se pressure existed, by which selenite turns to be transformed into fitted low toxic valence Se²⁻. Accordingly, by stationary phase *S. cerevisiae*, QDs with strong fluorescence emission can be synthesized massively after the following Cd-treated procedure.

1.2. Effect of selenite concentration

To metalize selenite, there are two competing pathways taking place in bacteria³⁷. An assimilatory pathway leads to produce organoselenium and a slow detoxification pathway results in generating Se^0 . When the concentration of selenite was high, detoxification pathway becomes predominant, which will metabolize selenite into almost 100% Se^0 fast³⁸. However, when the concentration of selenite was low, organoselenium will be produced with the same rate as that of protein synthesis, although Se^0 concentration also consumingly increased^{37,38}. As a result, in order to maximize organoselenium product and improve QDs' yield, Se concentration should be optimized.

As shown in figure 2c and 2d, yeast cells had the strongest abilities to manufacture QDs in 5mM Na_2SeO_3 , and the fluorescence intensities were also outstanding when the concentrations of Na_2SeO_3 were at 2, 3 and 7 mM respectively, which were probably resulted from the highly transformation of selenite to the selenium precursor for QDs' synthesis. When the concentration of selenite is gradually boosting up, toxic selenite will induce yeast to start assimilatory pathway, causing high-performed generation of organoselenium. Thus, fluorescence intensities increased at first with the increment of Na_2SeO_3 , but they did not exhibit linearity at the range of 2 mM to 5 mM Na_2SeO_3 . High concentrations of Na_2SeO_3 will aggravate detoxification pathway of yeast, and transform selenite into Se^0 dramatically. Besides that, high concentrations of Na_2SeO_3 generate reactive oxygen species (ROS) and bring about oxidative stress³⁹ and stress responses⁴⁰, which will reduce cell vitality and enzymatic activity, compromising the decrease of QDs' biomanufacture at last.

1.3. Effect of selenite incubating duration

After introduction of selenite, some bacteria such as *Ralstonia metallidurans* will uptake it slowly in the first place, during which bacteria will contain Se^0 and organoselenium in nearly equivalent proportions. However, after a long term incubation, most of the selenite will return to Se^0 finally³⁷. The organoselenium, if present, cannot be detected. In short, after introduction of selenite with appropriate concentration, the concentration of organoselenium will increase at the beginning and low ultimately. Hence, Se incubating duration was chosen from 12 h to 26 h to carefully analyze the best time point to obtain more QDs product with good fluorescence luminance.

As shown in figure 2e and 2f, the intensity of fluorescence peak reached maximum when Se incubating duration was 22 h. The weakening of fluorescence intensities over 22 h incubation arose possibly after decrement of organoselenium transformed into Se^0 or gradually transported from cytoplasm into vacuole²². The key point of 22 h was just in the time and space coupling. With the pressure of CdCl_2 onto the bacterial cells which have been treated with selenite at the scheduled moment (time coupling), the generated $\text{Cd}(\text{SG})_2$ would react exactly with organoselenium to compose CdSe QDs in cytoplasm just before transporting selenium into vacuole (space coupling).

1.4. Effect of cadmium concentration

The working concentration of cadmium was another important factor for attaining good QDs biosynthesis. As shown in figure 2g and 2h, the optimum concentration of cadmium sources was 1.0 mmol/L. These potency of bacteria in generation of CdSe QDs cut down significantly either at low cadmium concentration (0.5 mM) or at high cadmium concentration (5.0 mM).

Cytotoxicity of cadmium on the number of cells which have been pre-treated with selenite was first progressed. Bacterial optical density at 560 nm (OD_{560}) was measured to exam the number change of the bacteria cells. With the increment of cadmium concentration from 0.5 mM to 5.0 mM, yeast number lost however slightly (figure 2h). The reason for lessening maximum fluorescent intensity at high cadmium concentrations was therefore mainly lying on the toxicity of Cd, which was responsible for inhibition of enzyme activity and cell growth of yeast. On the other hand, Li et al²⁰ had demonstrated that GSH, which is interestingly induced by low concentration of CdCl_2 instead of Na_2SeO_3 ³³, was correlated with the biosynthesis of CdSe QDs. Since the increment of intracellular GSH had synergistic effect on CdSe QD formation in some bacterial species. CdCl_2 , therefore, is not only one of the reactants in reaction 4, but also a promoter of the formation of CdSe QDs.

Alternative synthesis method, which was named as "One Pot" method was also carried out. Na_2SeO_3 and CdCl_2 were introduced into broth simultaneously when bacteria were cultured to late log phase and stationary phase (24 h and 28 h) respectively. And then, the mixture was co-incubated for 24 h before harvesting. However, they only got weak fluorescent intensities in bacteria (Figure 2a, A and B).

In all, based on the work above, the optimum biosynthetic route could be deployed as following: yeast cells at stationary phase (28 h culturing) were co-cultured with 5mM Na_2SeO_3 for 22 h. After being harvested, yeast pellets were resuspended in YPD supplemented with 1 mM CdCl_2 and finally cultivated in dark for 14–44 h to obtain desired CdSe QDs with the fluorescence colors from green to red.

1.5. Effects of cadmium incubating duration on fluorescence emission peaks of CdSe

Particle sizes and fluorescence emission peaks of QDs synthesized by chemical methods can be tunable by changing the incubating duration. While biosynthesized in *S. cerevisiae*, CdSe QDs with different particle sizes and emission wavelengths are also capable of adjusting by controlling the duration of incubation with CdCl_2 . In the work, *S. cerevisiae* was treated according to optimum biosynthetic route and finally cultivated with CdCl_2 in dark for 14 h, 26 h and 44 h, respectively. As shown in figure 3, CdSe QDs with different colors were successfully obtained. When the incubating duration extended from 14 h to 44 h, the color of CdSe QDs under fluorescent microscope shifted from green to yellow, and then to red. TEM images (figure 4a) of QDs inside the yeast cells after 26 h CdCl_2 incubation confirmed that the size

distribution of QDs ranged from 15 to 20 nm with a typical compact particle center of CdSe QDs and a loose protein out-layer, which was bigger than the core size of their chemically synthesized counterparts (1.2 nm to 11.5 nm)⁴¹. Proteins and oligopeptides rich in yeast cytoplasm are supported to provide the enveloped layer outside the QDs. The following UV spectra of isolated CdSe QDs (Figure 4b) were consistent with the theory by showing two specific absorption peaks, peak a of ca. 280 nm proteins' absorption and peak b of the first quantum confinement absorption. The EDS result (Figure 4c) as well as XRD outcome (SI Figure 3) of the CdSe QDs confirmed that the QDs contained elements cadmium, selenium, oxygen, carbon and sulphur. The existing elements oxygen, carbon and sulphur which are the main integral of proteins and oligopeptides took a step closer to back up the hypothesis.

CdSe QDs were manufactured by the assistance of intracellular protein molecules and further on the protein layer gave the CdSe products with virtue of good water-dispersed and highly photostability. Separated from cells and detected by an inverted fluorescence microscope (figure 5), CdSe QDs demonstrated well dispersivity and high luminance. The fluorescent colors of the products were green and yellow which had their maximum peak wavelengths at 506 nm and 562 nm respectively. Compared with previous published work of both chemistry method (450 to 650 nm)⁴² and biological method (520 to ca. 560 nm and then to ca. 670 nm)²², the emitting wavelengths in the work however had a narrower tunable wavelength range. Moreover, some other comparisons of QDs products between the work and previous reports' were listed in SI Table 1. It is worthy mentioned that the fluorescence intensity of fluorescing cells had little change or some enhancement after being placed at 4 °C for two months or at room temperature for two weeks (Data not shown).

2. Biosynthesized CdSe QDs providing low cytotoxicity compared with hydrothermally synthesized TGA-capped CdSe and TGA-capped CdTe

MTT assay was performed to investigate the cytotoxicity of the biosynthesized CdSe QDs as well as that of the hydrothermally synthesized TGA-capped CdSe²⁸ and the TGA-capped CdTe QDs⁴³, which were typical hydrothermally synthesized QDs and usually used as positive controls. A wide range of QDs concentrations (100–2000 nmol of CdSe/mL) were chosen, as in figure 6e. Biosynthesized CdSe QDs lost cell viability in a dose-dependent manner, and statistically significant ($P < 0.05$) change was observed in 200 nmol/L and above compared with blank controls. Treated with the biosynthesized CdSe QDs, cell viability decreased less than that of hydrothermally synthesized CdSe and CdTe treated ones. They indeed didn't show obvious cytotoxicity with concentrations lower than 500 nmol/L which will make them a outperformed potential fluorescent probe in biological utility..

As shown in figure 6(a-d), after incubation for 48 h with INS-1 cells, both hydrothermally synthesized CdSe and CdTe QDs (c, d, 200 nM) caused some cells loosely attaching, rounding and blebbing, while 200 nmol/L biosynthesized CdSe

QDs (b) and the control (a) maintained much better cell morphology. Little morphological change was observed suggested the low cytotoxicity of biosynthesized CdSe QDs, which accorded with the results of MTT assay, shown in figure 6(e).

Conclusions

Five main factors during biosynthesis of CdSe QDs using *S. cerevisiae* as a matrix were systemically investigated by quickly measuring the maximum fluorescence intensities of QDs-contained bacteria solutions to determine the optimal synthetic conditions. The time point of adding selenite, the optimal working concentrations of the selenite and cadmium, the fittest incubating duration of selenite and cadmium altogether determined the biosynthesis efficiency of CdSe QDs. Herein, the particle sizes and fluorescence emission peaks of QDs had been tunable by controlling incubation duration of the yeast cells with CdCl₂. QDs of green and yellow fluorescent colors with corresponding maximum emission peaks at 506 nm and 562 nm respectively were isolated and proved to be protein-encapsulated and well-dispersive. Owing to the advantages of clean synthetic technology, simple experimental operation and endogenous biological modification, biosynthesis of QDs provided an alternative technique besides organic synthesis route and aqueous synthesis method. According to MTT assay and morphology observation, biosynthesized CdSe QDs showed much lower cytotoxicity compared with hydrothermally synthesized TGA-capped CdSe and TGA capped-CdTe. This method for CdSe QDs biomanufacture have been confirmed as a simple, environment friendly and repeatable way to produce highly hydrophilic and less toxic QDs, which will probably be qualified for using in cell labeling, pathogen tracking and bioimaging.

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Notes and references

1. U. Resch-Genger, M. Grabolle, S. Cavaliere-Jaricot, R. Nitschke and T. Nann, *Nat Meth*, 2008, **5**, 763-775.
2. S. K. Nune, P. Gunda, B. K. Majeti, P. K. Thallapally and M. L. Forrest, *Advanced drug delivery reviews*, 2011, **63**, 876-885.
3. L.-H. Xiong, R. Cui, Z.-L. Zhang, X. Yu, Z. Xie, Y.-B. Shi and D.-W. Pang, *ACS nano*, 2014.

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Journal Name

4. I. L. Medintz, A. R. Clapp, H. Mattoussi, E. R. Goldman, B. Fisher and J. M. Mauro, *Nature materials*, 2003, **2**, 630-638.
5. G. Palui, F. Aldeek, W. Wang and H. Mattoussi, *Chem. Soc. Rev.*, 2015, **44**, 193-227.
6. H. Zhu, N. Song and T. Lian, *J. Am. Chem. Soc.*, 2010, **132**, 15038-15045.
7. J. Huang, D. Stockwell, Z. Huang, D. L. Mohler and T. Lian, *J. Am. Chem. Soc.*, 2008, **130**, 5632-5633.
8. L. Li, A. Pandey, D. J. Werder, B. P. Khanal, J. M. Pietryga and V. I. Klimov, *J. Am. Chem. Soc.*, 2011, **133**, 1176-1179.
9. B. Chen, H. Zhong, W. Zhang, Z. a. Tan, Y. Li, C. Yu, T. Zhai, Y. Bando, S. Yang and B. Zou, *Adv. Funct. Mater.*, 2012, **22**, 2081-2088.
10. J. R. Lloyd, J. M. Byrne and V. S. Coker, *Curr. Opin. Biotechnol.*, 2011, **22**, 509-515.
11. K. B. Narayanan and N. Sakthivel, *Adv. Colloid Interface Sci.*, 2010, **156**, 1-13.
12. J. P. Monrás, V. Díaz, D. Bravo, R. A. Montes, T. G. Chasteen, I. O. Osorio-Román, C. C. Vásquez and J. M. Pérez-Donoso, *PLoS One*, 2012, **7**, e48657.
13. C. Mi, Y. Wang, J. Zhang, H. Huang, L. Xu, S. Wang, X. Fang, J. Fang, C. Mao and S. Xu, *J. Biotechnol.*, 2011, **153**, 125-132.
14. H. J. Bai, Z. M. Zhang, Y. Guo and G. E. Yang, *Colloids and Surfaces B: Biointerfaces*, 2009, **70**, 142-146.
15. P. Williams, E. Keshavarz-Moore and P. Dunnill, *J. Biotechnol.*, 1996, **48**, 259-267.
16. A. Ahmad, P. Mukherjee, D. Mandal, S. Senapati, M. I. Khan, R. Kumar and M. Sastry, *J. Am. Chem. Soc.*, 2002, **124**, 12108-12109.
17. H. Bao, Z. Lu, X. Cui, Y. Qiao, J. Guo, J. M. Anderson and C. M. Li, *Acta biomaterialia*, 2010, **6**, 3534-3541.
18. H. Bao, N. Hao, Y. Yang and D. Zhao, *Nano Research*, 2010, **3**, 481-489.
19. A. Syed and A. Ahmad, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2013, **106**, 41-47.
20. Y. Li, R. Cui, P. Zhang, B.-B. Chen, Z.-Q. Tian, L. Li, B. Hu, D.-W. Pang and Z.-X. Xie, *ACS nano*, 2013, **7**, 2240-2248.
21. P. Mohanpuria, N. K. Rana and S. K. Yadav, *J. Nanopart. Res.*, 2008, **10**, 507-517.
22. R. Cui, H. H. Liu, H. Y. Xie, Z. L. Zhang, Y. R. Yang, D. W. Pang, Z. X. Xie, B. B. Chen, B. Hu and P. Shen, *Adv. Funct. Mater.*, 2009, **19**, 2359-2364.
23. S. Prévéral, L. Gayet, C. Moldes, J. Hoffmann, S. Mounicou, A. Gruet, F. Reynaud, R. Lobinski, J.-M. Verbavatz, A. Vavasseur and C. Forestier, *J. Biol. Chem.*, 2009, **284**, 4936-4943.
24. N. Krumov, S. Oder, I. Perner-Nochta, A. Angelov and C. Posten, *J. Biotechnol.*, 2007, **132**, 481-486.
25. C. T. Dameron and D. R. Winge, *Trends Biotechnol.*, 1990, **8**, 3-6.
26. S. Cui, H. Chen, H. Zhu, J. Tian, X. Chi, Z. Qian, S. Achilefu and Y. Gu, *J. Mater. Chem.*, 2012, **22**, 4861-4873.
27. J. Pan and S.-S. Feng, *Biomaterials*, 2009, **30**, 1176-1183.
28. A. L. Rogach, A. Kornowski, M. Gao, A. Eychmüller and H. Weller, *The Journal of Physical Chemistry B*, 1999, **103**, 3065-3069.
29. H. Zhang, L. Wang, H. Xiong, L. Hu, B. Yang and W. Li, *Adv. Mater.*, 2003, **15**, 1712-1715.
30. J. Kessi and K. W. Hanselmann, *J. Biol. Chem.*, 2004, **279**, 50662-50669.
31. A. Tarze, M. Dauplais, I. Grigoras, M. Lazard, N.-T. Ha-Duong, F. Barbier, S. Blanquet and P. Plateau, *J. Biol. Chem.*, 2007, **282**, 8759-8767.
32. J. R. Pedrajas, E. Kosmidou, A. Miranda-Vizuete, J.-Å. Gustafsson, A. P. Wright and G. Spyrou, *J. Biol. Chem.*, 1999, **274**, 6366-6373.
33. K. Vido, D. Spector, G. Lagniel, S. Lopez, M. B. Toledano and J. Labarre, *J. Biol. Chem.*, 2001, **276**, 8469-8474.
34. Z.-S. Li, Y.-P. Lu, R.-G. Zhen, M. Szczypka, D. J. Thiele and P. A. Rea, *Proc. Natl. Acad. Sci. U. S. A.*, 1997, **94**, 42-47.
35. J. Kessi, *Microbiology*, 2006, **152**, 731-743.
36. C. Garbisu, T. Ishii, T. Leighton and B. B. Buchanan, *Chem. Geol.*, 1996, **132**, 199-204.
37. G. Sarret, L. Avoscan, M. Carriere, R. Collins, N. Geoffroy, F. Carrot, J. Coves and B. Gouget, *Appl. Environ. Microbiol.*, 2005, **71**, 2331-2337.
38. V. Van Fleet-Stalder, T. G. Chasteen, I. J. Pickering, G. N. George and R. C. Prince, *Appl. Environ. Microbiol.*, 2000, **66**, 4849-4853.
39. M. Wallenberg, E. Olm, C. Hebert, M. Bjornstedt and A. Fernandes, *Biochem. J.*, 2010, **429**, 85-93.
40. C. J. Bohler, A. M. Raines and R. A. Sunde, *PLoS One*, 2014, **9**, e101408.
41. C. Murray, D. J. Norris and M. G. Bawendi, *J. Am. Chem. Soc.*, 1993, **115**, 8706-8715.
42. L. Qu and X. Peng, *J. Am. Chem. Soc.*, 2002, **124**, 2049-2055.
43. H. Zhang, L. Wang, H. Xiong, L. Hu, B. Yang and W. Li, *Adv. Mater.*, 2003, **15**, 1712-1715.

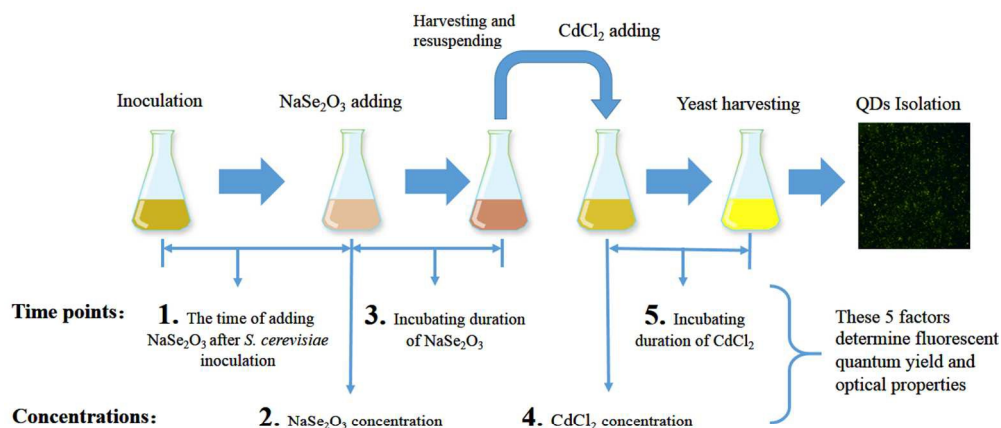


Figure 1. Schematic representation of the biosynthetic procedure of CdSe QDs by *S. cerevisiae*. The five crucial factors of biosynthesis include three key time points and two reagent concentrations in the synthetic process, which are pointed out and labeled as 1, 2, 3, 4 and 5 respectively.

338x190mm (96 x 96 DPI)

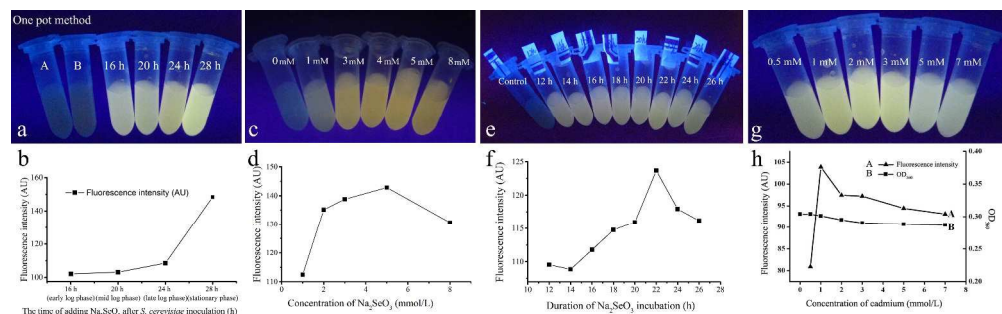


Figure 2. Optimized crucial factors during biosynthesis, maximum fluorescence intensity of yeast cells with the same optical density concentration at 560nm in one batch and photographs under 310 nm excitation unless otherwise definition.

a and b: fluorescence intensities influenced by time point of adding Na₂SeO₃. The Na₂SeO₃ was added at 16 h, 20 h, 24 h to 28 h's bacteria inoculation respectively. Here, tube A and B in photograph a were results synthesized by 'One pot' method, which was introduced with Na₂SeO₃ and CdCl₂ simultaneously when bacteria were cultured to late log phase and stationary phase respectively. The other were the results of 'Two-step' method.

c and d: fluorescence intensities of Na₂SeO₃ concentration affection, with the selenium concentrations from 0 (without the addition of selenium), 1, 3, 4, 5 to 8 mmol/L, respectively.

e and f: effect of Na₂SeO₃ incubating duration, with the incubating duration of selenium with matrices from 0, 12, 14, 16, 18, 20, 22, 24 to 26 h, respectively.

g and h: fluorescence intensities of harvested yeast (A) and their OD₅₆₀ (B) related to cadmium concentrations, where the concentrations of cadmium were 0.5, 1, 2, 3, 5 and 7 mmol/L from left to right with the same incubating duration of 26 h, respectively.

921x296mm (300 x 300 DPI)

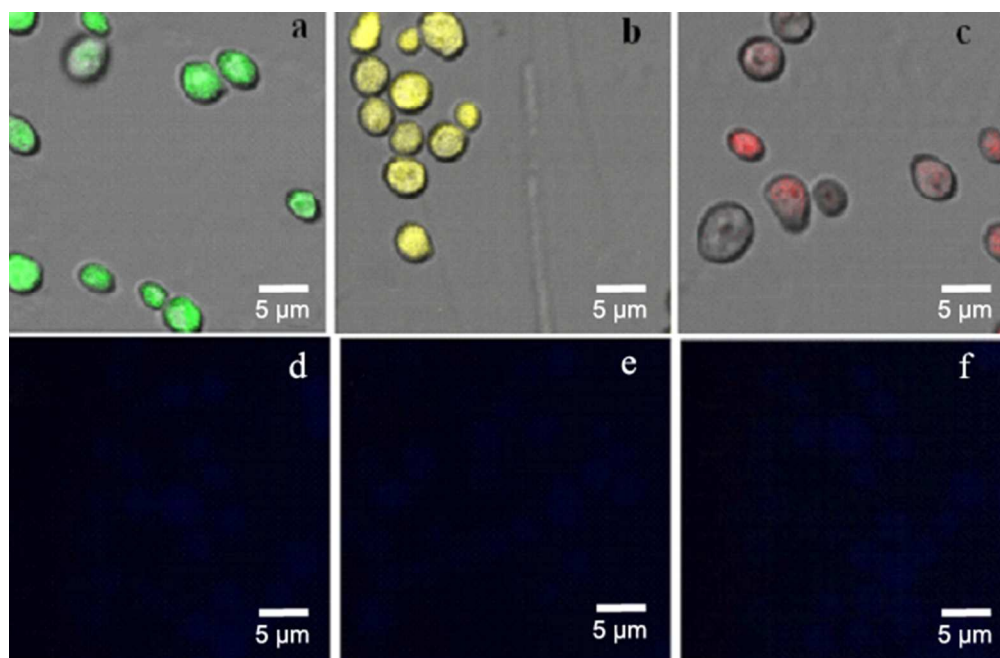


Figure 3. The confocal laser scanning images of yeast treated with different incubating duration of CdCl₂ (a, b, c) and images of controls treated only with selenium (d, e, f). The incubating durations were 14 h (a), 26 h (b), 44 h (c), 22 h (d), 24 h (e) and 44 h (f) respectively after yeast being treated with selenite.
173x113mm (96 x 96 DPI)

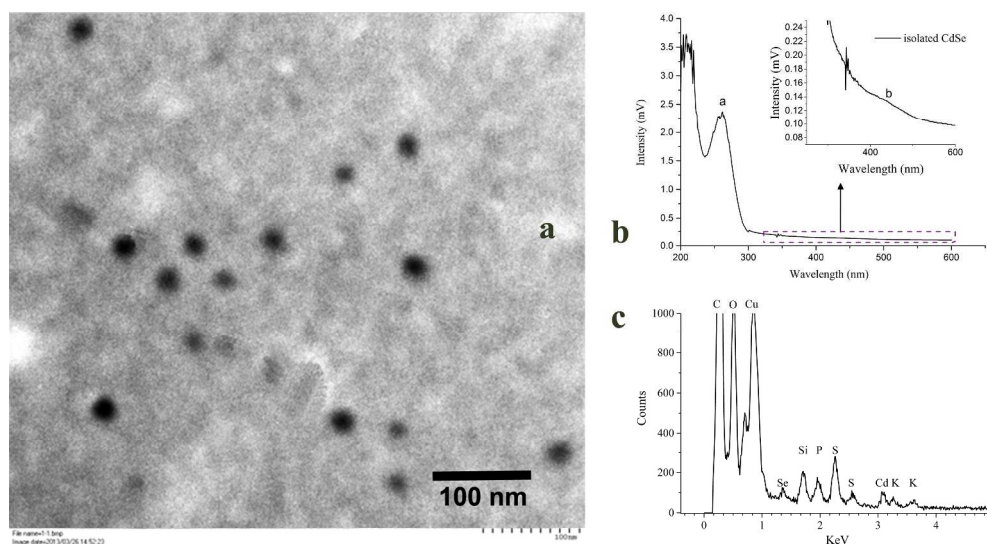


Figure 4. (a) Transmission electron microscopic image of QDs extracted from the stationary phase yeast cells (28 h cultured) treated with 5 mM Na_2SeO_3 for 22 h and 1 mM CdCl_2 for 26 h incubation. (Magnification= $\times 30.0\text{k}$; Acc.Voltage=80kV) (b) The absorption spectra of isolated CdSe QDs, obtained with the optimum biosynthetic route. The inset is partial enlargement. Peak a: absorption of proteins; peak b: first quantum confinement peak. (c) The EDS of isolated CdSe QDs. 493x263mm (200 x 200 DPI)

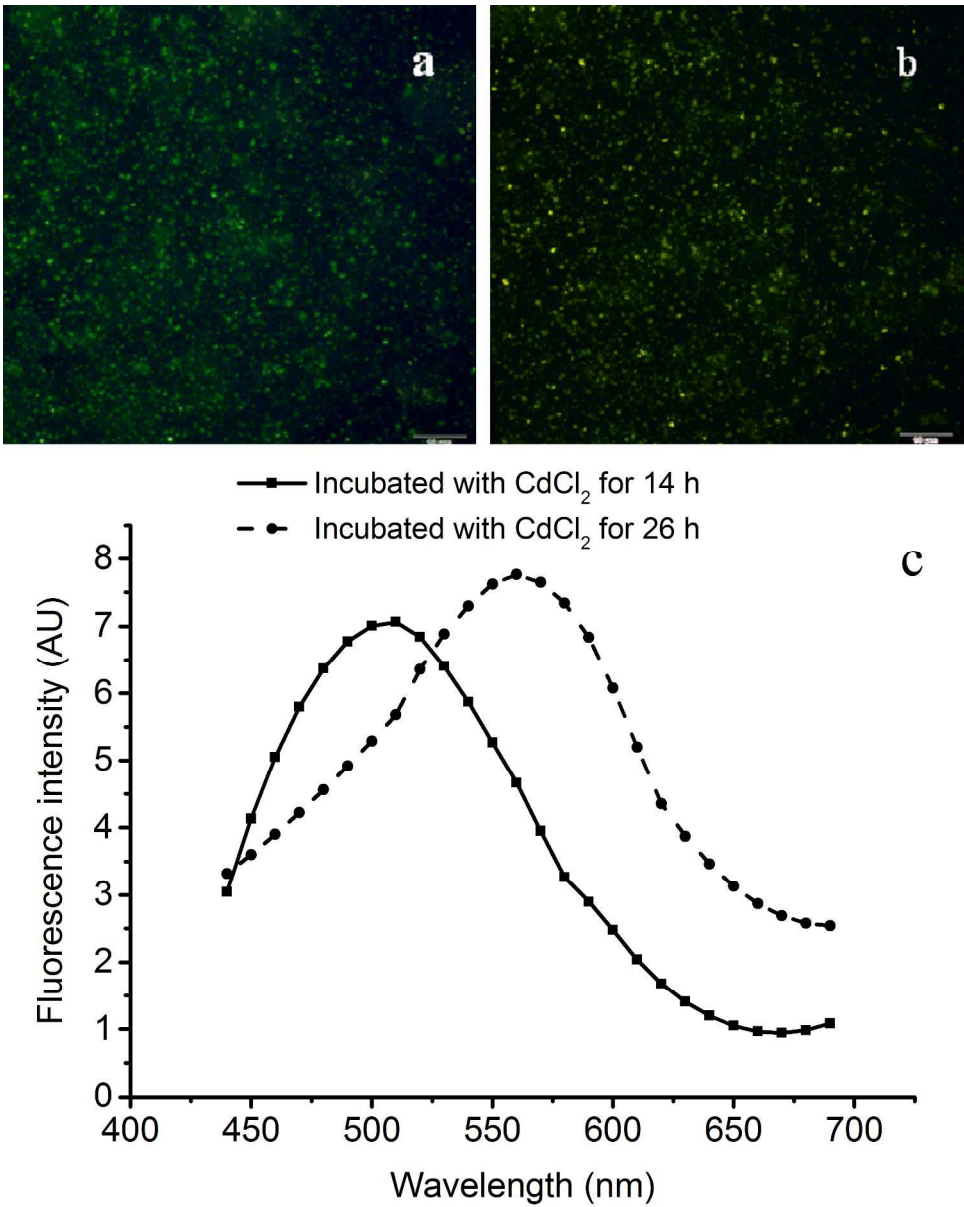


Figure 5. The inverted fluorescence microscopic images of QDs (a, b) isolated from above-mentioned yeast (Figure 3a and 3b) with different colors of fluorescence and their fluorescence spectra (c).
228x285mm (300 x 300 DPI)

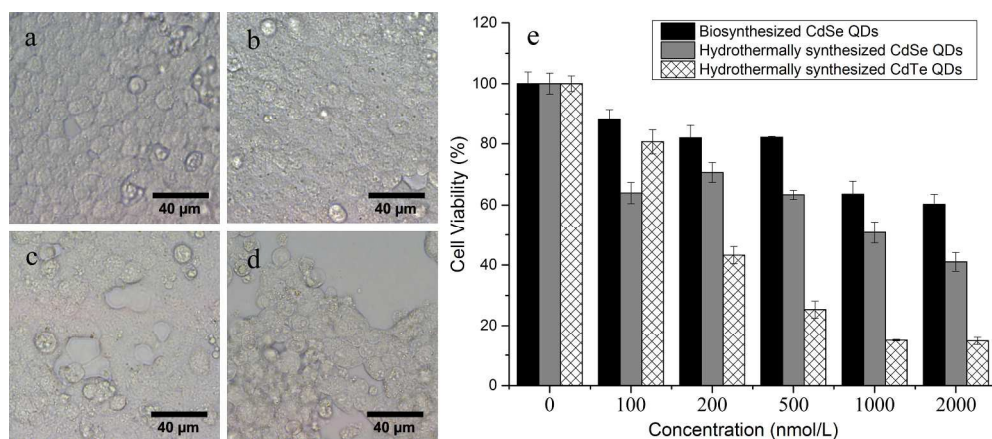


Figure 6. Morphology observation of INS-1 after incubated with PBS (a), biosynthesized CdSe QDs (b), hydrothermally synthesized CdSe QDs (c) and CdTe QDs (d) for 48 h at the concentration of 200 nmol/L (scale bar corresponds to 40 µm); MTT assay for MCF-7 cells treated with the above three kinds of QDs at different concentrations using untreated cells as controls (e). The results were expressed as means \pm SD from three independent experiments.
241x103mm (300 x 300 DPI)