CdTe/CdS-MPA quantum dots as fluorescent probes to label yeast cells: synthesis, characterization and conjugation with Concanavalin A

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ABSTRACT

Candida albicans is the most frequent human opportunistic pathogenic fungus and one of the most important causes of nosocomial infections. In fact, diagnosis of invasive candidiasis presents unique problems. The aim of this work was to evaluate, by fluorescence image analysis, cellular labeling of *C. albicans* with CdTe/CdS quantum dots conjugated or not to concanavalin A (ConA). Yeast cells were incubated with CdTe/CdS quantum dots (QD) stabilized with mercaptopropionic acid (MPA) (emission peak at 530 nm) for 1 hour. In the overall study we observed no morphological alterations. The fluorescence microscopic analysis of the yeast cells showed that the non-functionalized QDs do not label *C. albicans* cells, while for the QD conjugated to ConA the cells showed a fluorescence profile indicating that the membrane was preferentially marked. This profile was expected since Concanavalin A is a protein that binds specifically to terminal carbohydrate residues at the membrane cell surface. The results suggest that the QD-labeled Candida cells represent a promising tool to open new possibilities for a precise evaluation of fungal infections in pathological conditions.

Key words: biofilm, *Candida albicans*, candidiasis diagnosis, lectin, fluorescence microscopy

1. Introduction

Candida albicans is a commensal fungus of the human gastrointestinal and genitourinary tract that frequently causes superficial infections of mucosa and skin. The infection depends on imbalances between *C. albicans* virulence attributes and impaired host defense. In critically ill patients, this yeast may invade deeper tissues, penetrate the blood vessels, and cause life-threatening systemic infections[1].

Invasive candidiasis (IC) is a serious problem and associated with significant morbidity and mortality, so early identification of intensive care unit patients with signs of sepsis at high risk of IC is challenging[2]. Effective antifungal drugs are the gold standard treatment for IC, and success of the treatment depends on the speed in differential diagnostic of the yeast. Early diagnosis of IC and candidaemia is of vital importance mainly in immunosuppressed patients, however, blood cultures frequently lack sensitivity, are often inadequate in quantity and require 24–48 h of incubation[3]. Other techniques are also available but an efficient diagnostic method is still missing.

Quantum dots (QD) are semiconductor nanocrystals that have been applied as fluorescent probes. QD present unique optical properties including high photostability, broad absorption bands and size-tunable narrow emission spectra, which make them suitable for biological applications[4]. Beside, QD can be linked with biorecognition molecules such as proteins, peptides, and nucleic acids[5]. The major advantages of QDs are that they are brighter and do not suffer from photobleaching, a problem encountered on conventional fluorophores. This allows following processes in living cells on a long-time scale[6].

Lectins are proteins that selectively bind carbohydrates. Concanavalin A (ConA), a powerful agglutinating lectin that binds specifically to α-D-mannose and glucose, has been applied in new methodologies of diagnostic[7].

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In this work we explore the feasibility of CdTe/CdS-MPA nanocrystals as markers on living fungal cells and fungal biofilm, beside investigate ConA-QD conjugate molecules for labeling specific carbohydrates at the membrane cell surface and extracellular matrix of biofilm.

2. Materials and Methods

2.1 Strain and biofilm development

Candida albicans ATCC 90028 was sub-cultured from vial stock onto Sabouraud dextrose broth in aerobic conditions for 24 h at 37°C. Then, the cells were centrifuged and resuspended in phosphate buffer saline (PBS) at final concentration of 10^7 CFU/mL. For biofilm formation, *C. albicans* was suspended in fresh medium at concentration of 10^7 CFU/mL. Biofilms were cultured in polystyrene flat-bottom 96-well plates inoculated with 200 µL of yeast suspension during four days at 37°C. Medium was replaced every 48 h.

2.2 Quantum dots synthesis

A green-emitting cadmium telluride quantum dot (CdTe QD) aqueous solution was prepared by mixing a solution of cadmium perchlorate and mercaptopropionic acid (MPA) at pH=10.5. The system was maintained under argon atmosphere and Te^{-2} ions (obtained by the controlled reduction of Te with sodium borohydride) were added and the system refluxed at 80-90°C. The Cd:Te:MPA molar ration used was 2:1:4.6. The optical properties of the colloidal nanocrystals suspensions were obtained by electronic absorption, emission and excitation spectroscopy.

2.3 ConA-QD conjugate preparation

To use QD as an efficient target contrast agent for imaging cells, this particle required to be conjugated with specific biorecognition molecules. The lectin concanavalin A was chosen due to its interaction with carbohydrate[8], which are numerous on the yeast cell wall and biofilm. Furthermore, ConA also interacts with QD. The ConA-QD conjugate was prepared just before each experiment. The procedure was divided in four parts: quantum dots dilution, ConA dilution, pH adjustment and conjugation. Briefly, 800 µL of CdTe/CdS-MPA were carefully transferred to another tube and then 4 mL of deionized water was added resulting in a solution at 5,5 µM. In the second part, 0.028 g of ConA was diluted in 1 mL of 0.01M Phosphate Buffer, which was experimentally determined as appropriated proportion for ConA-QD conjugation. The pH of the QD was carefully adjusted, with addition of small drops of 0.1M HCl solution until pH 8. ConA-QD conjugate was prepared in two concentrations: CdTe/CdS-MPA with ConA at 0.28 mg/mL, and CdTe/CdS-MPA with ConA at 50 µg/mL. The reaction mixture, also called conjugation, was made by the addition of ConA in QD suspension (with pH adjusted), and the suspension was incubated for 2 h at 25ºC to allow total reaction of the compounds.

2.4 Incubation with *C. albicans*

After bioconjugation, QD-ConA was incubated with planktonic and biofilm cells of *C. albicans*. QD-ConA was added to yeast suspension in a proportion of 1:1 (v/v), and kept in the dark for 1 hour at room temperature. After this period, the cells were centrifuged and washed in PBS. In biofilm experiment, 200 µL of QDs were incubated with it for one and half hour at room temperature, and then the biofilm was washed in PBS three times.

2.5 Cell imaging

Ten-µL aliquots were taken from the suspension with yeast cells and QD-ConA, and were placed on a slide and coverslip for analysis. The 96-well plates containing the biofilms were also imaged. A fluorescence microscope (Leica DMI4000B, Leica Mikroskopie und System GmBH, Germany) with excitation at 480 nm was used. The cells were observed with 63x objective and images at 512 x 512 pixels resolution were recorded. Fluorescence signals were collected in the green range (527/30 nm bandpass filter) from CdTe/CdS-MPA. The false output color green was superimposed for the figures.

3. Results and Discussion

Figure 1A shows the absorbance of the CdTe/CdS-MPA that presents a long absorption band from ultraviolet until 500 nm. Electronic excitation (left) and emission profiles of QD and ConA-QD (right) are displayed in figure 1B. We observe that the maximum excitation is at λ= 469 nm and that emission profiles of the conjugate and non-conjugate QD are identical, except for a decrease in the emission intensity due to dilution and decline in the pH of the original QD suspension. We also observe a symmetrical band centered at λ= 530 nm with a very low-intensity tail ranging up to 650 nm. The emission band is credited to the electron-hole recombination and the tail to recombination in surface traps[9].

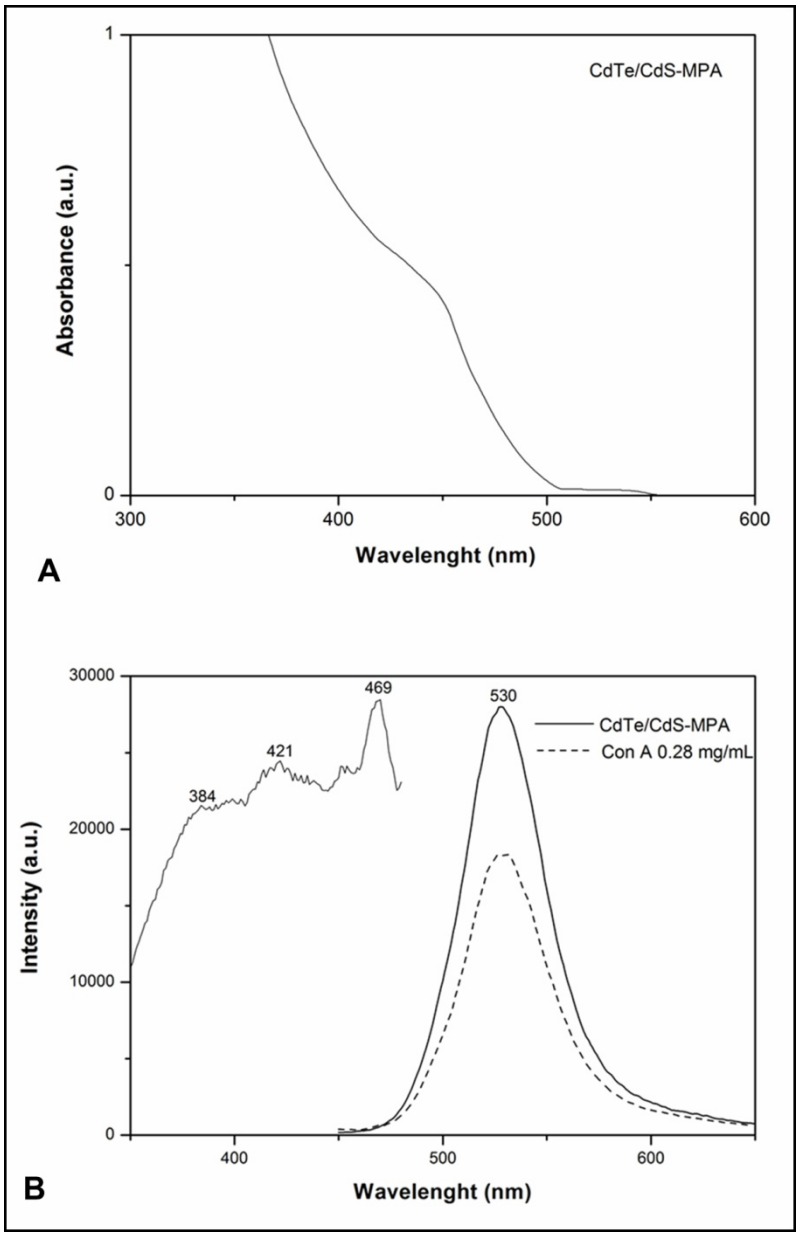

Figure 1: Absorption spectrum (A) and (B) electronic excitation (left) and emission profiles (right) of CdTe/CdS-MPA.

The imaging of *Candida albicans* cells presented a low intensity of autofluorescence. Green-emitting CdTe/CdS-MPA QD conjugated or non-conjugated to ConA were observed in fluorescence microscopy of 24-hour culture of yeast cells. QD labeling has not affected the growth and the cell division as well as development and morphology have not been altered. Figure 2 displays yeast cells imaging using quantum dots bioconjugated with lectin (ConA) or non-conjugated. We observe that the non-conjugated QD do not label *C. albicans* cells (figs. 2A and 2B), while for the QD conjugated to ConA, the cells showed an intense fluorescence suggesting that the cell wall was preferentially labeled (figs. 2C and 2D). This contour was expected since ConA is a protein that binds specifically to terminal carbohydrate residues localized at the cell wall[7]. Besides, non-functionalized QDs do not label *C. albicans* cells (Fig 2A).

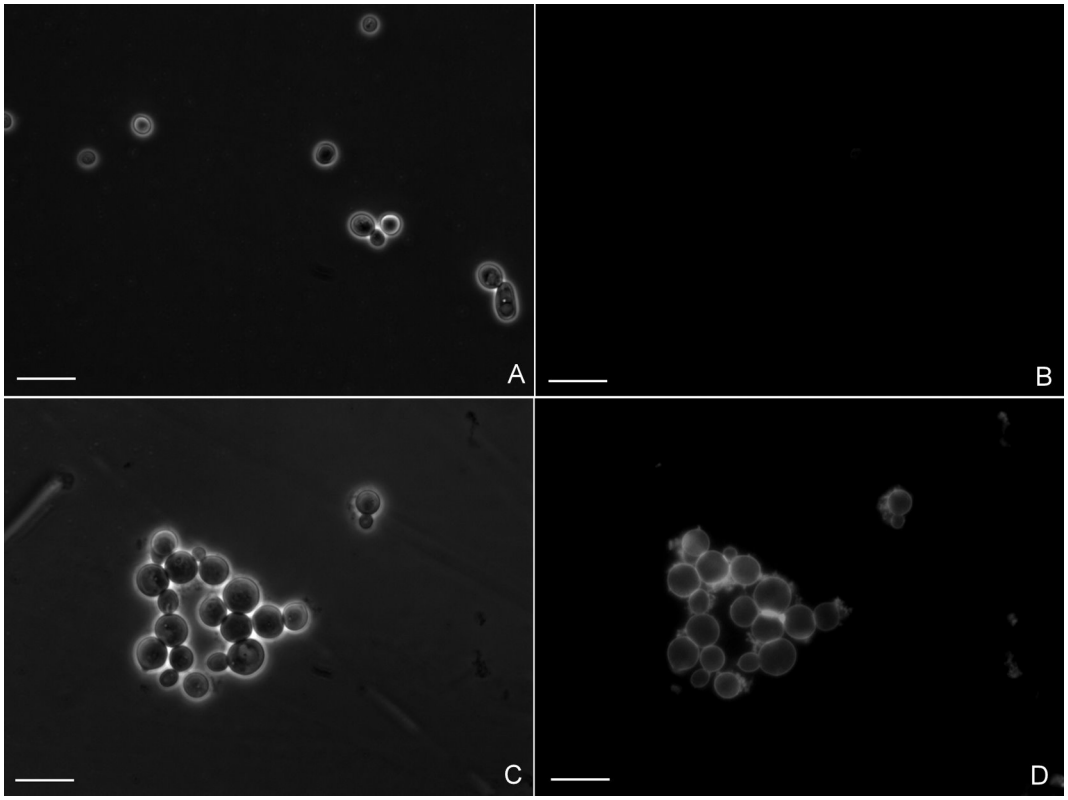

Figura 2: Fluorescence microscopy images of *C. albicans* cells. We present two images, transmission and fluorescence, from the same field for each compound used. (A) transmission and (B) fluorescence show the cells incubated with CdTe/CdS-MPA. (C) transmission and (D) (fluorescence) illustrate cells incubated with CdTe/CdS-MPA conjugated to ConA. Excitation was made at λ= 480/40 nm. Scale bars represent 10 µm.

To increase the challenge of labeling fungal cells, we developed *Candida albicans* biofilm during 4 days. At this stage, biofilm shows an aggregate of fungi embedded in an organized polymeric extracellular matrix, which assume different forms such as micro-tubes adhered to surfaces. Particularly, *C. albicans* biofilms have a highly heterogeneous architecture composed of cellular and non cellular elements[10].

Figure 3 displays biofilm images captured by fluorescence microscopy. Differently of our previous result, quantum dots did not preferentially label cell surfaces but bound to extracellular polymeric substances (EPS) in the biofilm matrix. This finding fits with those reported by Morrow *et al.* about the association of quantum dot nanoparticles with *Pseudomonas aeruginosa* biofilm[11]. Furthermore, the binding of ConA to biofilms does not necessarily prove the presence of specific target sugars in the extracellular polymeric substances (EPS) in biofilms. ConA may bind to non-EPS targets or adhere nonspecifically to components of the biofilm matrix[12].

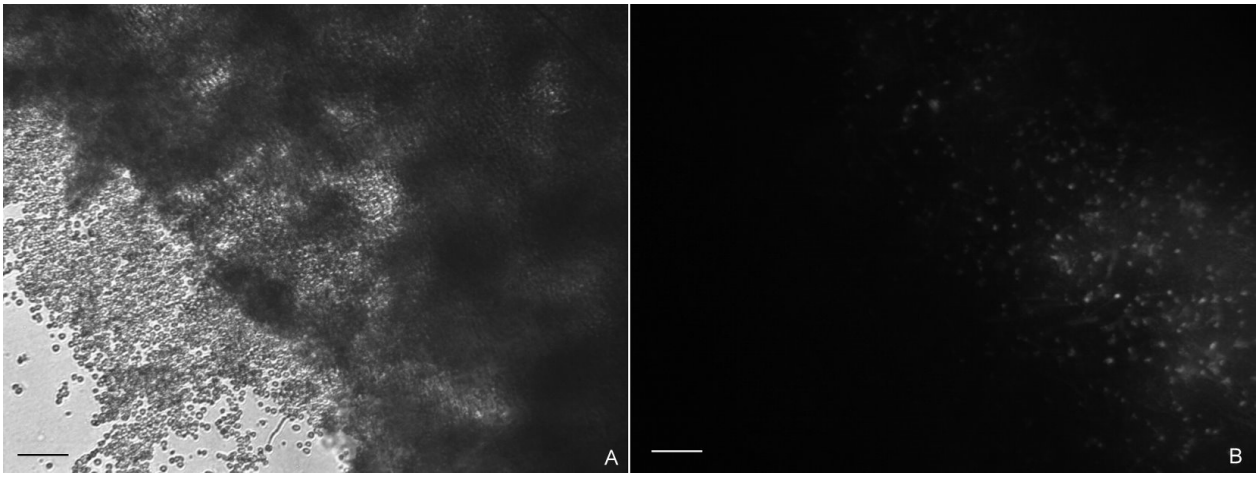

Figura 3: Fluorescence microscopy images of *C. albicans* biofilm cultured during 4 days. (A) transmission and (B) fluorescence show biofilm incubated with ConA-CdTe/CdS-MPA conjugate. Excitation was made at λ= 480 nm. Scale bars represent 200 µm.

4. Conclusion

In summary, our results show the possibility of using a physiological fluorescent marker to label living yeast cells. It is the first time that QD have been used as biomarkers for the study of *Candida albicans*. At the moment, we are investigating QD conjugated to biorecognition molecules emitting red fluorescence as a potential marker of *C. albicans* in order to develop an effective diagnostic method for candidiasis.

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