Chapter 6

Biocompatible Magic Sized Quantum Dots: Luminescent Markers and Probes

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Additional information is available at the end of the chapter

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Abstract

Nanoscience and nanobiotechnology have aroused great academic and technological interest. Works relating biomaterials at the nanoscale can reach new biotechnologies and help in the development and use of tools for bioimage and diagnosis applications. In this work we demonstrated the advantages of magic sized quantum dots as luminescent markers and probes to bioimage applications. The visualization of MSQDs bioconjugated with biological probes in cells were performed at periods greater than 2 h, and visualization with no commercial dye would not be possible. Therefore, we demonstrated that theses biocompatible nanocrystals are luminescent markers and probes to diagnosis.

Keywords: nanocrystals, biocompatibility, luminescence, bioconjugation, probes

1. Introduction

Nanotechnology in life sciences has as one of its objectives to diagnose diseases in a precise, fast and effective way, offering improvements to the existing diagnostic methods. In this sense, several nanomaterials have been studied and developed to benefit the biomedical technologies.

One of the nanomaterials with high potential for application in nanomedicine is the quantum dot, which is a semiconductor nanoparticle. This nanoparticle is a promising material for the diagnosis and understanding of cell biology, being used both for image generation and for detection of signals through [1–3].

Measures based on fluorescence have biochemical specificity and high sensitivity. There is considerable interest in the use of quantum dots as inorganic fluorophores because they have significant advantages over conventionally used fluorescent markers. For this, it is necessary that these nanoparticles are biocompatible so that they are conjugated properly to biological molecules, by means of bioconjugation techniques [4–7].

In cell biology it has been a practice to use fluorescent labels such as organic dyes, coordinating compounds of lanthanide ions and recombinant fluorescent proteins [8–12]. Organic fluorophores are used as molecular markers to identify structural compartments, molecules, or organelles. These fluorophores can be linked (conjugated) to the target directly or indirectly through conjugation with antibodies and other molecules that bind secondarily to the target. The fluorophore DAPI (4'6-diamidino-2-phenylindole), for example, has affinity for DNA molecules, and is commonly employed for labeling in cell nuclei.

Although they are widely used in the labeling of cellular structures, tissues and organisms, some limitations of these fluorophores should be considered. An irreversible photochemical reaction common to fluorophores is the photobleaching or photodegradation caused by the photochemical destruction of the fluorophore, causing them to irreversibly lose their fluorescence, either after being excited or after a few minutes, depending on the intensity of the source of excitation [13–15].

In this way, samples stained with such dyes need to be kept in dark environments and the microscopy section needs to be performed quickly, making it difficult to monitor a whole cellular process. In addition to photodegradation, other disadvantages involved in these types of labeling are: high toxicity and cell death using ultraviolet (UV) [16–18]. Fluorescent proteins such as GFP (Green Fluorescent Protein) are apparently non-toxic, but may induce undesired cellular processes, and it is a laborious technique.

Due to the disadvantages in the use of conventional fluorophores, several researches have been directed towards the development and reproducibility in the synthesis of nanocrystals for use in bio detection and bioimaging [19–21]. In particular, quantum dots (QDs) have been extensively studied because they exhibit high absorption and the absorption and emission spectra are easily controlled as a function of size, shape and crystalline phase [15, 19, 22], this enables tuning of the emission length as well as high efficiency. Like this, multicolored images can be obtained with the same material when it has different diameters [19, 21, 23, 24].

Unlike most conventional dyes which have a narrow absorption spectrum, the QDs have the additional advantage that a single laser can excite luminescence at different wavelengths while each organic dye must be excited over a long length of single wave and well defined [25–29].

The studies and applications of QDs reveal their efficacy as new luminescent probes for diagnostic purposes, enabling real-time acquisition of a single cell surface receptor, as well as the development of non-invasive models for the detection of small tumors [30]. Another highlight is the application of the QDs in living cells and tissues. Depending on the binder attached to

the quantum dots, they can be injected into cells by the micro injection technique. Once inside the cells, the QDs biochemical reaction [27, 31–33].

Recently, it has been shown that the magic-sized quantum dots (MSQDs) present great advantages in the medical and biological area. These MSQDs are quantum dots with extremely small sizes (<2 nm) and physical properties that are completely different from those presented by QDs [34, 35]. These MSQDs present thermodynamically stable structures, wide luminescence range, great size stability over time, relatively narrow absorption spectra, and heterogenic growth [36, 37]. The term magic size is related to a (magic) number of atoms in the structure that makes MSQDs extremely stable. The wide luminescence spectrum occurs because MSQDs have internal atomic defects [38].

The MSQDs can be important tools in the investigation of metastases and have been proposed as vehicles for administering drugs that can initiate certain photoactivated chemical reactions due to highly stable luminescence over time [39]. The cultures of cells marked with MSQDs continue to maintain their functions, remaining viable for analysis for prolonged periods of time [39–42].

In this chapter we highlight the advantages of MSQDs as a biocompatible luminescent marker and its use as a specific probe. In the biological labeling assays, we use CdSe/CdS MSQDs bioconjugated with Pepstatin A, BnSP-6, BthTx-II and a specific BC Fab antibody to allow its tracking within the cell and, in addition, to verify the specificity of this treatment for breast cancer cells.

2. Magic quantum dots luminescent markers and probes

Figure 1a shows the absorption and emission spectra of CdSe/CdSe MSQDs that observed a narrow band around 428 nm and a broad emission spectrum, respectively [41]. This broad emission is characteristics of the MSQDs and that is of great applicability in biological processes, enabling their visualization in different detection channels, varying from blue to red (**Figure 1a**) [43]. Using the three detection filters, the fluorescence images of the MSQDs in the cells were recorded (**Figure 1b–d**) [39–41].

One of the major problems of organic fluorophores besides cytotoxicity is the duration of luminescence, making it impossible to trace and observe biological assays as a function of time. Quantum dots present high intensity of luminescence compared to dyes and a monitoring time greater than 2 h, but after this time is observed a drastic decrease of luminescence intensity, that difficult of monitoring. This occurs because QDs interact with cellular proteins and decrease the luminescence intensity over time [17]. However, the high stability of MSQDS allows the highly stable luminescence, even after 36 h, (**Figure 2**) [39].

The fluorescence images of the CdSe MSQDs in HeLa cells after (a) 24 h and (b) 36 h are shown in **Figure 2**. It is verified that even after 36 h it is possible to visualize the luminescence of MSQDs. In **Figure 2(c)** it is shown that the observed luminescence is due to a set of MSQDs and that they were constructed with the CdSe core, a thin layer of CdS and functionalized with hydroxyl groups. These results show that the MSQDs can be used as biocompatible luminescent markers [39].

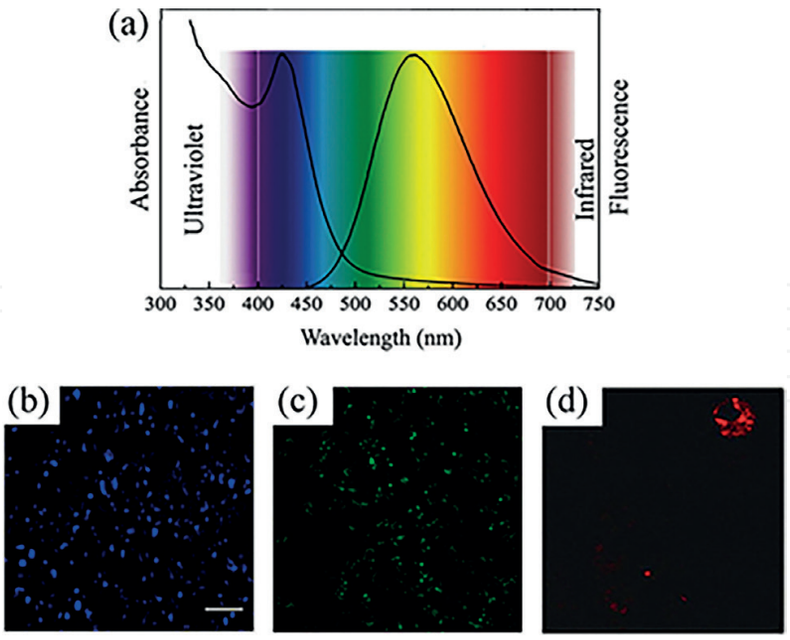

Figure 1. (a) Absorption and fluorescence spectra of CdSe/CdS MSQDs, (b)–(d) fluorescence images of MSQDS in cells using the blue, green and red detection filters, respectively [39–41].

Therefore, the photostable MSQDs allow researchers to investigate the distinct cellular process, in the form of experiments with phagocytosis and vesicles intracellular traffic [44]. Regarding in vivo experiments, stable luminescence MSQDs could be used for tumor cells migration since the monitoring could be done for days.

It is crucial the high biocompatibility of MSQDs, in other words, they cannot be harmful to the cells. It is well known that MSQDs have cadmium ions (Cd^{2+}) adsorbed in their superficies. The manipulation of the composition and diameter of core and shell of MSQDs reduce the cellular contact with Cd^{2+}. In this scenario, Cd^{2+} could stimulate various noxious effects as apoptosis, necrosis and DNA damage by the induction of reactive oxygen species production. Such effects extremely change the biology of the cells. For example, in an experiment with phagocytosis and intracellular traffic, apoptotic cells present a deficient phagocytose process which can infer misunderstand able results [39].

To investigate the true cause of the cytotoxic effect of cadmium chalcogenides QDs, we synthesized CdSe MSQDs with different amounts of Cd^{2+} adsorbed on their surface and have shown that this quantity is directly related to cytotoxicity (**Figure 3 a,b**). The biocompatibility is increased with decrease of Cd^{2+} adsorbed on MSQDs surface (**Figure 3b**). In addition, the decrease of Cd^{2+} is confirmed by the lower expression of the protein (**Figure 3c**). Thus, based on this, we developed new synthesis methodologies to develop a CdS shell using the Cd^{2+} adsorbed [45, 46]. Thus, we have shown in our recent studies that this shell increases biocompatibility and has no immunological effects [41].

Figure 4 shows fluorescence images of CdSe/CdSe MSQDs alone and bioconjugated Pepstatin A, BnSP-6, BthTx-II and a specific BC Fab antibody in the MDA-MB-23.1 [40, 41]. The fluorescence from MSQDs bioconjugated was detected inside the cells' nuclei compartment of MDA-MB-231. Notably, we verified that the MSQDs alone were not internalized in MDA-MB-231 (**Figure 4a**) [40].

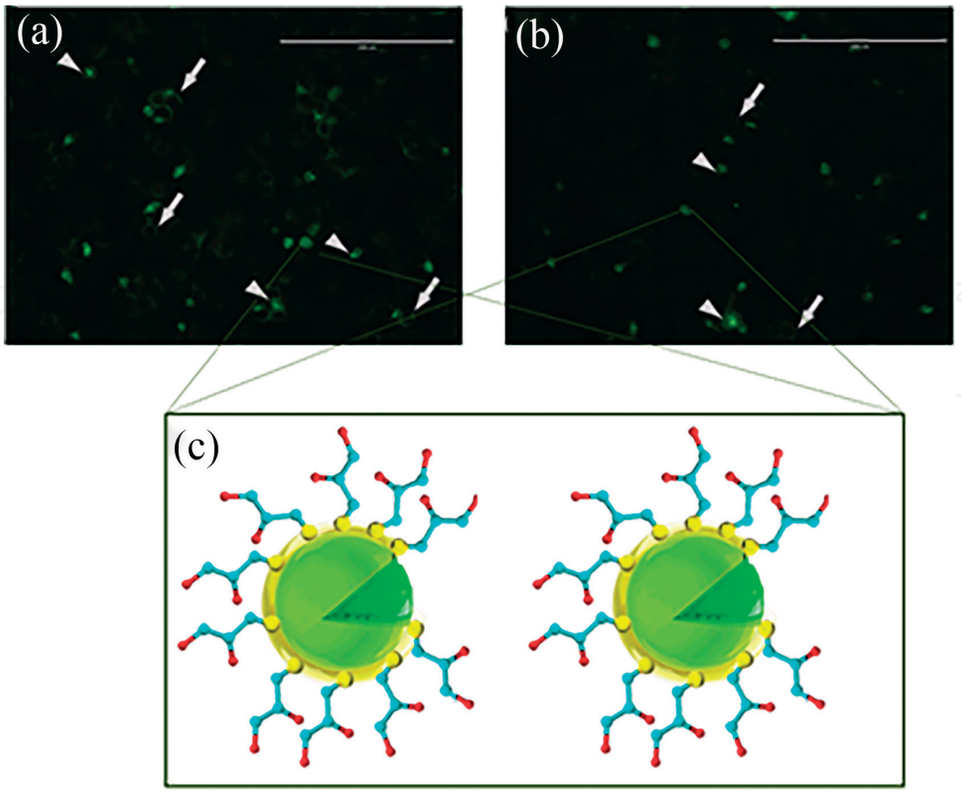

Figure 2. Fluorescent image of incubation with 0.05 M of MSQDs (a) after 24 h and (b) after 36 h. White arrow indicates MSQDs interacted to cellular membrane. Arrows head indicates MSQDs inside the cells. Scale bar = 200 μm. (c) Representation of a set of MSQDs [39].

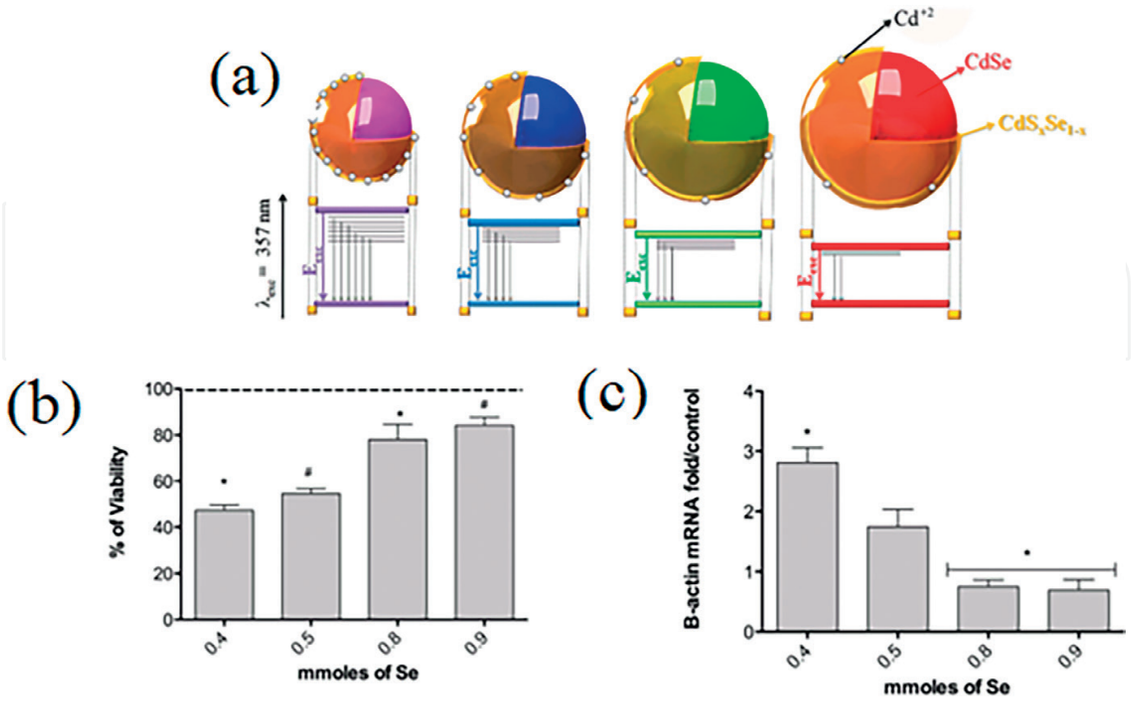

Figure 3. (a) Representation of CdSe MSQDs with different amounts of Cd^{2+} adsorbed on their surface, (b) Viability in function of diminution of Cd^{2+} and (c) B-action mRNA fold/control in function of diminution of Cd^{2+} (increase of Se).

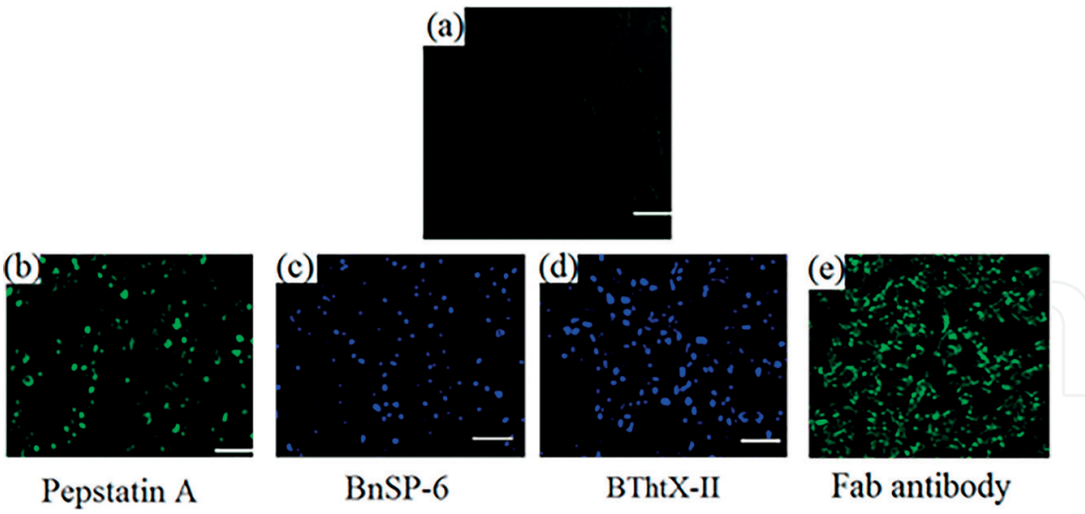

Figure 4. Fluorescence images of (a) CdSe/CdSe MSQDs, bioconjugated (b) Pepstatin in 6 h after treatment, (c) BnSP-6 in 3 h after treatment, (d) BthTx-II in 3 h after treatment and (e) a specific BC Fab antibody in the MDA-MB-23.1 [40, 41].

This result confirms the bioconjugation of the MSQDs with biological probes and in addition, all the assays were performed at periods greater than 2 h, and visualization with no commercial dye would not be possible. Therefore, the group presents experience in the development of new biocompatible luminescent markers specific to each type of treatment.

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