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Quantum dots as a sensor for quantitative visualization of surface charges on single living cells with nano-scale resolution

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ABSTRACT

We developed a technique using quantum dot (QD) as a sensor for quantitative visualization of the surface charge on biological cells with nano-scale resolution. The QD system was designed and synthesized using amino modified CdSe/ZnS nanoparticles. In a specially designed buffer solution, they are positively charged and can homogeneously disperse in the aqueous environment to label all the negative charges on the surfaces of living cells. Using a wide-field optical sectioning microscopy to achieve 2D/3D imaging of the QD-labeled cells, we determined the charge densities of different kinds of cells from normal to mutant ones. The information about the surface charge distribution is significant in evaluating the structure, function, biological behavior and even malignant transformation of cells.

1. Introduction

It is well known that mammalian cells exhibit a net negative charge following the ionization of the chemical groups of cell membrane proteins/macromolecules. The surface charge of a cell plays an important role in maintaining cell membrane stability; affecting cell-structure/functions (Ando et al., 1999; Chen et al., 2007; Dobrzynska et al., 1999; Garcia et al., 2000; Knowles et al., 1994; Nordenström, 1985; Romero et al., 1997; Simpson and O'Neill, 2003), even intracellular molecules (Kang et al., 2008); and controlling cell adhesion, aggregation, cell–ions, cell–virus, antigen–antibody, and cell–drug interactions (Meiselman et al., 1999; Rogers et al., 1992). Some abnormal cells such as tumor cells, were also found to change their surface charges accompanied with their mutation in structure and functions during its malignant transformation (Nordenström, 1985). Therefore, detection of a cell's surface charges and their distribution is of considerable significance in evaluating the cell's structure/functions, and predicting the cell's biological behavior, especially, its malignant transformation.

At present, the most common way to detect a cell's surface charge is to measure its electrophoretic mobility (EPM) or Zeta potential using different kinds of electrophoresis. Charge density of the cell membrane can be deduced from the Zeta potential by considering the ionic strength of the cell suspension (Cook et al., 1961; Durocher et al., 1975; Gottschalk, 1960). However, this method cannot provide information about the charge distribution on the cell. Neither can the technique of photometric titration (Thethi et al., 1997). Although the cytochemical techniques using different electronic microscopic markers (Gasic et al., 1968) can evaluate the charge distribution of fixed cells under electronic microscope, no currently available technique can quantitatively visualize the charge distribution on living cells.

Therefore, it is strongly desired to have such a technique for the research in biomedicine. To approach this problem, one possible way is to use fluorescence probes to detect the surface charges. However, there are some difficulties in using conventional fluorescence probes to visualize the surface charge on living cells. The method usually requires specific conjugation of fluorescence probes with the target receptors on a cell membrane. Thus only some parts of the charges can be labeled. Moreover, it is difficult to obtain quantitative information using conventional fluorophores because their fluorescence only last for a short time and attenuate quickly.

The technique of quantum dot (QD) brings us the possibility to image all the surface charges and their distribution on the surface of a living biological cell. QDs have advantages over the conventional fluorophores such as brighter fluorescence, resistance to photobleaching, narrow emission spectrum and broad excitation spectra. Being modified with amino or capped with dihydroxylipoic acid, they can be hydrophilic and charged either positively or negatively thus can bind to all the identities with opposite charge on the sur-

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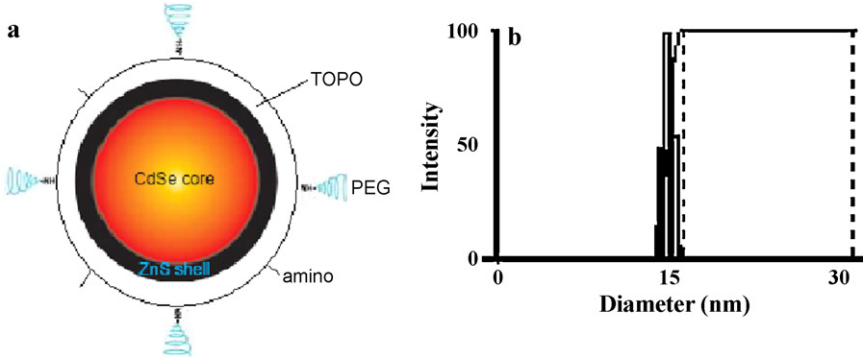

Fig. 1. The structure of the amino modified CdSe/ZnS core–shell QD (a) and the size distribution of the QDs (b) in the aqueous environment of living cells. The averaged diameter of the QD is 15.0 ± 0.8 nm.

face of a cell by electrostatic interaction (Jaiswal and Simon, 2004). However, there are some challenges. For example, the modification may decrease the stability of QDs and their quantum yield. The colloidal nature of QDs in aqueous environment also makes them susceptible to irreversible aggregation. Therefore, the QDs should be synthesized with these considerations to improve their stability in cellular environments. At the same time, a suitable buffer solution has to be designed to ensure that the QDs can avoid aggregation and label all the surface charges on the cells in the solution.

Another challenge is to find a fast 3D imaging technique to visualize the surface charge of a QD-labeled living cell. To obtain 3D visualization, one has to perform image scanning on the cell. At present, laser confocal scanning microscopy is the most common way to perform 3D image scanning. However, this technique requires 2D point-by-point scanning in the lateral direction and then layer-by-layer scanning in the axial direction. The point-by-point scanning in the lateral direction takes time, especially when high resolution imaging is requested. Though QD can last its fluorescence for longer time than common fluorescence probes, its intensity may still change considerably during the period of a 3D scanning by a laser confocal scanning microscopy. Therefore, a faster 3D scanning technology should be employed to perform 3D quantitative visualization on the surface charges of living cells.

Accordingly, we developed a technique using QD sensing system combined with wide-field optical sectioning microscopy for 2D and 3D visualization of the surface charges on living biological cells. The QD system includes a CdSe/ZnS core–shell biocompatible nanoparticle QD and a specially designed buffer solution. The QD with CdSe/ZnS core–shell was chosen as the sensor due to its narrow emission bands which spans the whole optical spectrum and its steady fluorescence lasting for a certain period of time. In the specially designed buffer solution, the QDs are positively charged and homogeneously dispersed so that they can label all the negative charges on living cells. Using the wide-field optical sectioning microscopy to achieve 2D/3D imaging of the QD-labeled cells, we determined the charge densities of different kinds of cells from normal to mutant ones.

2. Materials and methods

The QDs provided by Wuhan Jiayuan QDs Company Limited had an average grain size of 3.52 nm. They were modified by a modular ligand based on poly(ethylene glycol) (PEG) coupled with amino to enhance the stability and biocompatibility (Fig. 1a). After the modification, the QDs were positively charged and had a diameter of 15 nm (Fig. 1b).

To avoid aggregation of the QDs in the aqueous environment of biological cells, we designed a buffer system by mixing 5 ml 0.9% sodium chloride solution and 10 ml 5% glucose solution in the volume ratio of 1:2. The QDs were proved to be stably dispersed in the buffer solution by dynamic light scattering measurement (Fig. 1b). The solution was also proved to be able to keep different kinds of biological cells alive.

Owing to its ability of obtaining 2D image at video rate or even faster, a wide-field fluorescence microscopy was employed as the optical sectioning technique to achieve 3D scanning of a living cell within a period in which the QD fluorescence intensity was without notable change. The wide-field fluorescence microscopy was built based on a Nikon TE300 inverted fluorescence microscope (Huang, 2005a). It can perform both bright field and fluorescence optical sectioning, and complete sectioning (20–30 layers) on a biological cell within 10 s. Therefore, by combining this microscopy with the QD sensing techniques, it is possible to quantitatively visualize the surface charge distribution of a living cell with nano-scale resolution.

Considering that RBC is a relatively simple cellular system without intracellular membrane and organelles but its membrane exhibits functional activities that are representative of various plasma membranes, we used human RBC as the model for the QD labeling and imaging. The RBCs were separated from the fresh venous bloods taken from 16 young, healthy volunteers (aged 23–26). The young and senescent RBCs were fractionated by density gradient centrifugation on Percoll gradient (Murphy, 1973), washed twice in PBS and then kept at 4 °C for subsequent use. Studies on human blood (providing informed written consent) were approved by Ji Nan University Animal Care and Use Committee conforming to the Chinese Public Health Service Policy on Human Care and Use of Laboratory Animals.

To label the surface charges of the RBCs, the QD was dissolved in a 0.1 M PBS solution (PH 7.4, at 37 °C) with a concentration of 4.88 μmol/l. Then 1 μl of the QD solution was added to 200 μl of the previously mentioned buffer solution with RBCs. The mixture was shook gently, rested for at least 10 min and then washed three times to get rid of those QDs which did not bind to the cells. By performing the above steps, the QDs were assumed to disperse homogenously and firmly label all the surface negative charges on the cells. Due to their size and chemical nature, QDs were unable to diffuse through cell membrane (Jaiswal and Simon, 2004). Therefore, only the surface charges of the cells were labeled. Just before labeling, the cells were treated with 0.1% glutaraldehyde so that they can be labeled with QDs and at the same time keep alive with flexibility (Liu and Huang, 2006).

The fluorescence images of the QD-labeled RBC were visualized and captured by the wide-field fluorescence scanning microscope (Huang, 2005a) with an excitation light at 388 nm and the emission at 588 nm. To obtain thin optical sections of the specimen, two wide-field optical sectioning techniques were implemented. The first one used structured illumination which was achieved by