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Biological applications of Semiconductor Nanoparticles

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Outline:

Introduction

Bionanotechnology is one of the key technologies of the 21st century. It has attracted the attention of many scientists, due to the vast applications that can be produced by merging material science and biotechnology. This field involves the utilisation of biological systems such as cells, cellular components, and proteins, to manufacture efficient nanostructures (1–5). Nanotechnology is the new utensil that explores biomolecular structures, functions and properties. Bionanotechnology have already been used to measure structural elements of cells, molecular recognition and also have been applied in drug delivery (6–12). Biomolecules and biological complexes are naturally existing building blocks that have a great importance in the direction of molecular recognition and self-assembly. More complex structures such as viruses and bacteriophages could be assembled at the nanoscale. Such bio-nanostructures can direct the "bottom up" assembly (13–17).
In the same vein, the unique electronic, optical, and catalytic properties of nanoparticles associate in the generation of new devices and material with new functions and properties (18–21). Additionally, the conjugation of nanoparticles with biomaterials may render new magnitudes in nano-biotechnology. Biomolecules have a size range between 2-100 nm. These proportions are similar to those of the nanoparticles which increase the possibility of their applications into living cells (22–26). Figure 14.1 below shows the dimensions of nanoparticles compared with other species.
Nanomaterials have at least one spatial dimension in the range of 1-1000 nm, and show unique mesoscopic properties that will be discussed later in this chapter. Additionally, semiconductor nanoparticles (NPs) or also called nanocrystals (NCs) are characterised as small inorganic solids that have a size range of 1-100 nm. Figure 14.1 below displays the dimension of different species showing the position of the nanoparticles in between (18,27–29).

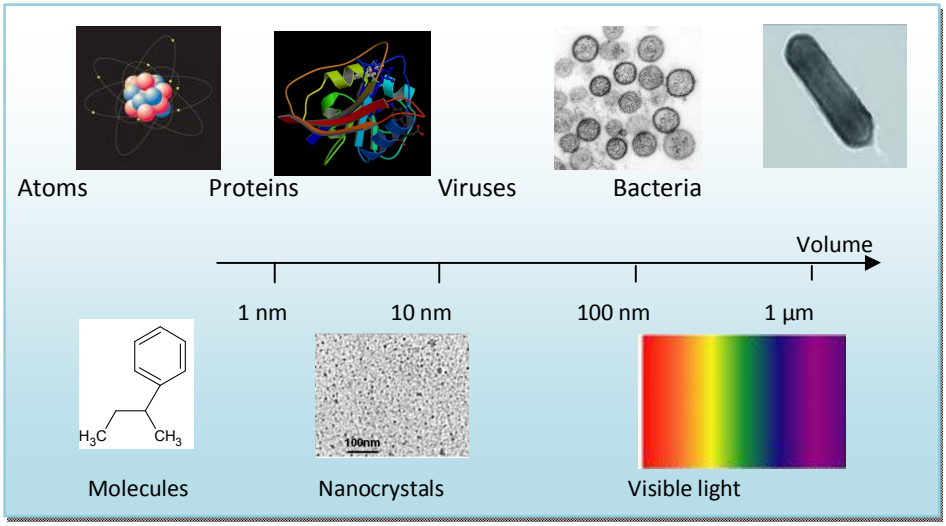

FIGURE 14.1
dimensions of different species showing the position of nanomaterials

In this chapter we will be discussing a specific type of semiconductor nanoparticles called Quantum dots (QDs). QDs are highly luminescent, colloidal semiconductor nanocrystals. QDs have unique size-dependant properties, which make them highly attractive for applications in catalysis, phosphors,

photovoltics, light emitting diodes (LEDs) and biological labeling. The main appealing feature of semiconductor NCs, are their mesoscopic properties that differentiates them from bulk crystals. The mesoscopic properties will be discussed in details later in this chapter (4,29–37).

When quantum dots (QDs) were first explored 20 years ago, they were applied in electronics and optics. At that time, it was still unrealized that QDs could be suitable for applications in biology and medicine. However, their use as research devices has extended prominently in the last decade, and currently QDs are being used as probes for high resolution molecular imaging of cellular components and for tracking cell activities and movements.
Besides, it is possible to bind quantum dots to proteins and receptors to check with which molecules they interact and to explore their location in the cell. Hence, QDs are used in biomedical applications because of their unique tuneable optical properties, (38–40). The semiconductor QDs are usually synthesized in organic media, which will produce hydrophobic QDs, in order to use these QDs in biological applications they should be rendered biocompatible; by masking their surface with a hydrophilic coating. The key approaches to make QDs biocompatible include silanisation and surface exchange with bifunctional molecules (38–41). Consequently, forming nanobioconjugates should be synthesised in a way that keeps the bioconjugate in the nano size regime. (42).Typically, the nanoparticles are coupled to the biomaterials through two main approaches; direct covalent linkage and non-covalent interactions between the particle and biomolecules (43). QDs of different emission wavelengths could be excited with a single light source, which gives these particles an advantage to be used for multiplex imaging of molecular targets coating layer of wider band gap semiconductor plays an important role to passivate its surface, and reduce non-radiative carrier recombination and thus increases the optical characteristics of the semiconducting nancrystal (20,27,44).

Properties of semiconductor nanoparticles

Optical properties

QDs are spherical semiconductor nanocrystals made of elements from the periodic groups II-VI (CdSe) or III-V (InP)(34,45). QDs are extremely fluorescent, due to their spatially confined band gaps resulting in physical, and optical, properties in-between compounds and single molecules. Quantum confinement allow QDs to emit light at different wavelengths directly related to their core diameter(46). Also, the relatively small size of QDs allows the crystal to behave as a single molecule with all its atoms being excited and emitting light together, resulting in a high signal intensity. In the same vein, one of the main optical properties of QDs is their resistance to photobleaching, and their long fluorescence lifetime when compared with organic flourophores. Organic flourophores or organic dyes tend to decay within nanoseconds and exhibit photobleaching (24,31,47–49) figure 14.2.

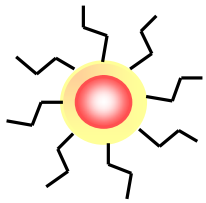

FIGURE 14.2
Florescent core/Shell quantum dot, with hydrophobic ligands on the surface

Additionally, quantum dots are made up of 100–2000 atoms. This unique type of nanocrystals has physical and chemical properties that are determined by two factors. The first factor is the high surface/ volume ratio of the nanoparticles. The second factor is the size of the particle, which determines the electronic and physical properties of the material. Yet, the optical spectra of nanocrystalline semiconductors (figure 14.3) exhibit a blue shift in their photoluminescence spectra as the particle size decreases. This size dependent optical property is an example of the size quantisation effect which occurs when the size of the nanoparticle is smaller than the bulk-exciton Bohr radius, *a*B, of the semiconductor (equation 1)(36).

$$a_B = \frac{\hbar^2 \in}{e^2}\left[\frac{1}{m^*_e} + \frac{1}{m^*_h}\right]$$

Equation 1

Where, a_B is the bulk-exciton Bohr radius, ϵ is the bulk optical dielectric coefficient, *e* the elementary charge, and me* and m_h* the effective mass of the electron and hole respectively.

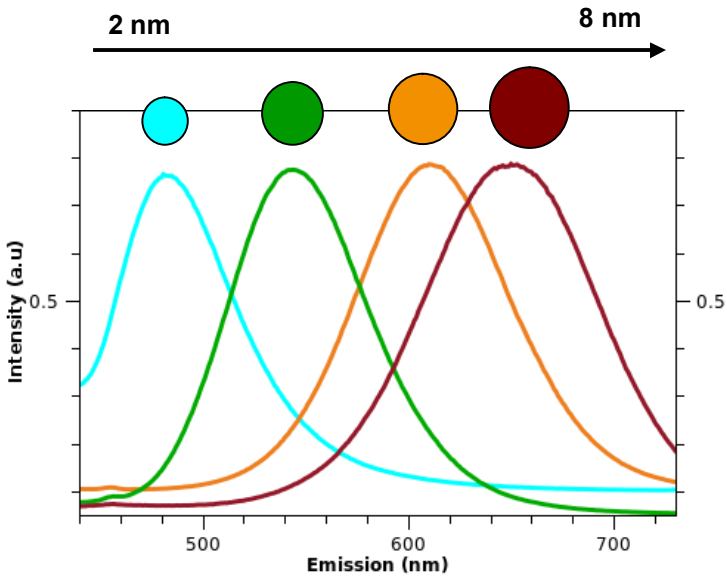

FIGURE 14.3
Emission spectra of differently colored InP/ZnS quantum dots (excited at 400 nm), displaying the increase in size near the IR region

Moreover, nanoparticles have very little scattering of visible light due to their small size. Mie's solution to maxwell's equations describes all aspects of light scattering. For spherical particles much smaller than the wavelength of light (0.1xλ) it can be simplified to the Rayleigh approximation. For Rayleigh scattering the intensity *I* of light scattered by a single small particle from a beam of nonpolarised light of wavelenghth λ and intensity I_0 is proportional to $(d/2)^6$ (equation 2).

$$I = I0 \frac{\left(1 + \cos^2 \theta\right)}{2R^2} \cdot \left(\frac{2\pi}{\lambda}\right)^4 \cdot \left(\frac{n^2 - 1}{n^2 + 2}\right)^2 \cdot \left(\frac{d}{2}\right)^6$$ **Equation 2**

Where *R* is the distance to the particle, *ϑ* is the scattering angle, *n* is the refractive index of the particle, and *d* is the diameter of the particle [].

Quantum confinement and quantum size effect

The optical absorption spectrum of semiconductor nanocrystals is a direct method for the assessment of quantum size effects. The absorption of a photon excites an electron from the valence band to the conduction band, which is associated with the band gap energy (*E*g). The absorption of photons with energy similar to that of the band gap, $h\nu \geq E$g, activates an optical transition that creates an electron in the conduction band of the semiconductor alongside with a hole in the valence band.
Absorption of photons with energy superior to *E*g leads to excitations exceeding the conduction band edge**.** The absorption (*A*) of light by a semiconductor material with thickness *l* can be expressed by an expression similar to the Beer law, where *α* represents the absorption coefficient of the solid and is a function of the radiation frequency (equation 3)

$$A = \alpha I$$ **Equation 3**

The electronic band structure of semiconducting nanoparticles is different from the bulk semiconductors, in an intrinsic semiconductor an electron (e^-) is excited into the conduction band by photon absorption, leaving a positively charged hole (h^+) in the valence band. When these two charges communicate, by their Coulumb potential developing a quasiparticle called an exciton, they are bound in a way that resembles the electron-proton binding in the hydrogen atom, these charges interact as the exciton recombines, excess energy is released either as photoluminescence (radiative decay, Kr) or heat (non-radiative decay) the equation 1-4 presents the exciton's Bohr radius (45,50–52).
Semiconductors have band gap energy (*Eg*) wich separates the valence and conduction bands. The density of bonding orbitals (σ) and antibonding orbitals (σ*) decreases as the size of the semiconductor decreases, which directs to a discrete energy level within the valence and conduction bands which are comparable to the discrete energy levels of individual atoms (Figure 14.4).

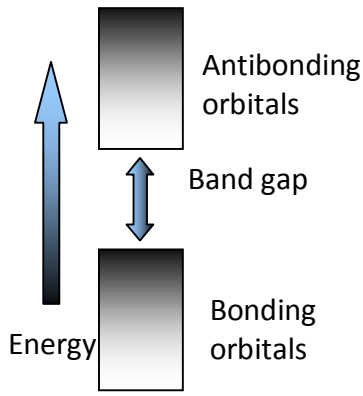

FIGURE 14.4
the fluorescence properties of quantum dots arise from the energy gap of the confined QD

The nanocrystal is in the weak confinement regime when the semiconductor radius *r* is larger than the exciton Bohr radius *aB*, in this case the quantisation of the exciton centre-of-mass motion take place and the exciton is competent to shift as a net uncharged particle. It is assumed that the nanocrystal is strongly confined when r<<*aB* (53). Furthermore, the strongly confined semiconductor nanocrystals have a size dependent photolomuniscence wavelength, the energy levels in a QD become quantised due to the confinement of electrons (figure 14.4 above), the band gap energy E_g, is determined by the orbitals inside the particle. The Brus equation below shows these changes as a function of nanocrystal size(Equation 4) (46,53,54).

$$E_g(r) = E_g(r = \infty) + \frac{h^2\pi^2}{2r^2\mu} - \frac{1.8e^2}{4\pi\varepsilon_r\varepsilon_0 r} \qquad \textbf{Equation 4}$$

Where r=radius of the nanocrystal, ε_r=the relative dielectric constant of the semiconductor and ε_0= vacuum permittivity.

Biological Applications of semiconductor nanoparticles

Nanosensors

Quantum dots (QDs) have attracted the attention of many scientists working in the field of biosensors. Their long-term photostability, makes real-time and continuous monitoring possible. Lately, scientists have focused on creating sensors for different target analytes, where QDs are utilised as electron donors for fluorescence resonance energy transfer (FRET) between a donor (the QDs) and an acceptor(49,55,56). In addition to FRET, QDs have many valuable properties that have been exploited for the development of nanosensors that depend on the alteration in the emission wavelength, voltage or fluorescence intensity. QDs have been used as optical sensors of ions (Cu^{2+}, Mn^{2+}, CO^{2+}, Hg^{2+}), drugs, organic pollutants such as polycyclic aromatic hydrocarbons, and small biological molecules (glucose, folic acid)(57–61).

Immunochromatographic assays

A promising model of nanosensors is the development of immunochromatographic test strips, to construct easy-handling nano-biosensors. Gold nanoparticles (GNPs) have formed the basis of an accessible tool already marketed; which is the home pregnancy test. The GNPs are used in such test strips because of their high stability compared with other systems that rely on the use of fluorescence or enzymatic labels. Also, the surface of GNPs makes them good candidates for rapid antibody–antigen recognitions. In the presence of the human chorionic gonadotropin hormone (HCG), a sandwich-type assay is formed between the secondary antibody–immobilized GNPs immunocomplex and the primary antibody immobilized on the membrane. When the antigen–antibody reaction takes place, the viewing window of the home pregnancy test, shows a red line caused by the aggregation of gold nanoparticle at that location. The red line is a visual indicator of the presence of the HCG hormone in the urine sample. Figure 14.5 below demonstrates the principel of the home pregnancy test based on the use of GNPs (62).

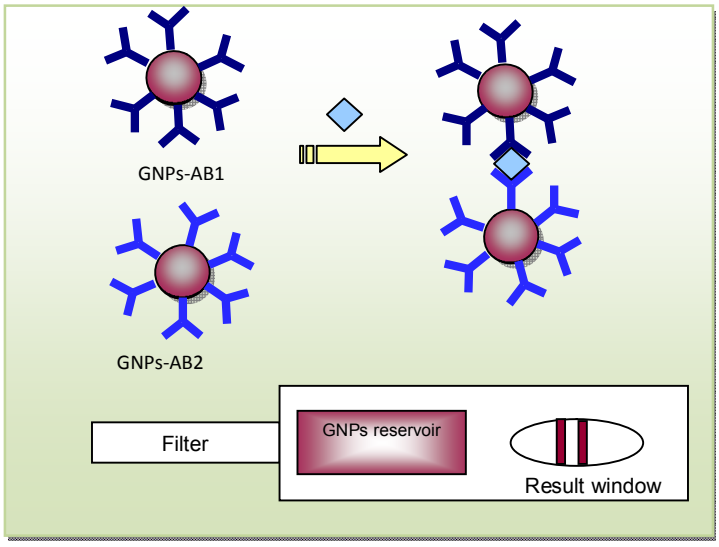

FIGURE 14.5
the use of gold nanoparticles (GNPs) in the home pregnancy test. The GNPs are conjugated to Antibodies that recognises the human chorionic gonadotropin hormone (HCG); Urine passes from the flow stick to a central reservoir containing gold nanoparticles. If pregnancy hormone is present, the GNPs form a complex with the HCG, which results in the aggregation of the nanoparticles, this aggregation is diplayed as a red signal in the viewing window of the test

Glucose nano-sensing

The detection of glucose is an important application of nanotechnology. Scientists have widely investigated this specific type of nanosensor. The function of the glucose nanosensor is based on the presence of highly fluorescent nanoparticles, QDs. The quantification of glucose concentrations is of high importance in controlling different biotechnological processes, and in diagnosing several metabolic disorders, like diabetes. Previously, other glucose detection methods have been applied, like amperometric, spectrophotometric, fluorometric methods.
In the case of the QD-glucose sensor, the enzyme glucoseoxidase (GOX) is coupled to the nanoparticles to create an optical detection tool. In the presence of glucose the enzyme GOX releases Hydrogen Peroxide (H_2O_2) as a result of the enzymatic reaction; Hydrogen Peroxide initiates the formation of free radicals, which reduces the luminescence of the nanoparticles. The quenching of the fluorescence of QDs is a direct indicator of the presence of glucose in the system (63). Figure 14.7 below shows an example of fluorescent detection of glucose. Hydrophilic CdSe/ZnS QDs have been used to sense glucose. The fluorescence quenching of the QDs has been applied to measure the concentrations of glucose in aqueous solution. The quenching process was based on the transfer of electrons from the QDs to enzymes (glucose oxidase (GOD), peroxidase (HRP)), which catalyze the oxidation/reduction reactions of glucose and all of the components involved in the process. The QDs have been able to detect glucose by introducing them directly into the glucose solution after their conjugation with the GOX enzyme(58,64–67) (figures 14.6 and 14.7).

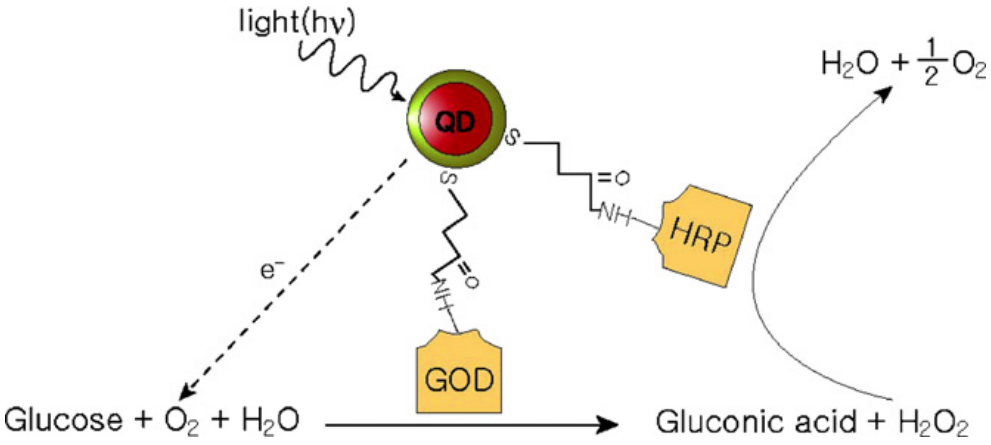

FIGURE 14.6
Optical sensing of Glucose. In the presence of Glucose, Hydrogen Peroxide is released and causes a rapid decrease in the photoluminescence of quantum dots from reference (64).

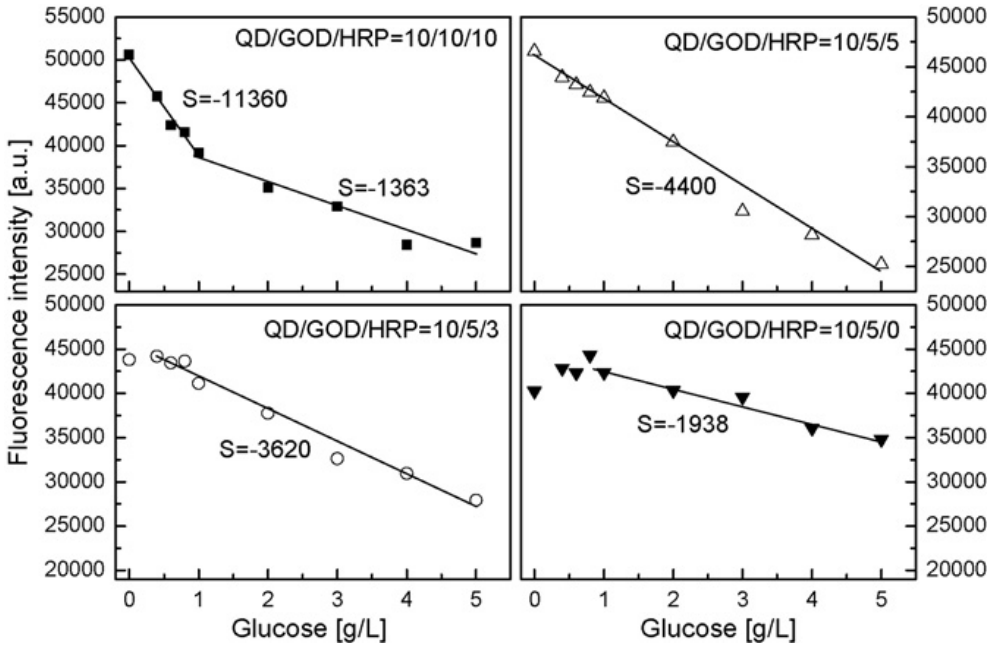

FIGURE 14.7
Change in fluorescence intensities of the QD-FRET-based probes at different glucose concentrations and various volume ratios of QDs/GOD/HRP added to glucose solution (64)

Live cell labeling

Quantum Dots have been projected as alternatives for traditional organic dyes and fluorescent proteins in imaging applications due to their exceptional photophysical properties. QDs are about 10–100 times brighter and show narrower and more symmetric emission spectra than organic fluorophores. Additionally, QDs present large absorption cross-sections, which make them 100–1000 times resistant against photobleaching. QDs with different emission wavelengths could be excited with a single light source, producing spectrum that ranges from the ultraviolet to the NIR (68–71).
Mainly, live cells studies with QDs have shed the light on the presence of cellular membrane markers. As they ease the access of QDs and do not require passage of the probes through the cell membrane. The next step is to target the cytoplasmic molecules. It has been really challenging to determine the best technique for cytoplasmic translocation of QDs. A variety of techniques have been extensively used for intracellular labelling with QDs. For instance: passive uptake, receptor-mediated and non-specific endocytosis, cell penetration, liposome mediated intracellular delivery, electroporation and microinjection (72–75). Electroporation of QDs into cells involves the application of electric pulses, which temporarily disturb the phospholipid bilayer, consequently, increasing the permeability of cellular membranes. Nevertheless, it may cause the aggregation of QDs inside the cells causing cell death. However, direct injection (micro-injection) is one of the most commonly applied methods of QDs translocation into living cells. Microinjection of QDs into cells, is a direct method that introduces QDs into the cytoplasm or the nucleus of the cell by applying pressure or electrical impulse. This method have made intracellular organelles labeling feasible and more specific, however, it is a time consuming and might cause the aggregation of the QDs due to the high pressure applied when applying this technique. Hence, scientists working in this field are highly encouraged to develop new techniques that could be less time-consuming and less aggressive on the QDs samples.
Jaiswal et al. *have* applied the QD cell labeling method to investigate the effect of starvation on *Dictyosteliumdiscoideum* cells. These cells were starved for different periods of time, and then they were labeled with differently colored QDs. Consequently the labeled cells were imaged; it has been observed that the QDs have the tendency to aggregate depending on the degree of starvation of cells. The experiments executed by Jaiswal et al. could not be achieved using the conventional organic dyes due to their susceptibility to photobleaching(76).
Moreover, Dubertret et al. has reported a very important study about the use of CdSe/ZnS Qds in bioimaging. PEG functionalized QDs were synthesized to study the development in *Xenopus* embryos(41) Figure 14.8. The QDs were internalized by microinjection into individual cells of the growing embryo; the embryonic development has been studied for individual cells, since the fluorescence of QDs was confined to the offspring of the studied cells. This study has revealed that QDs used were highly stable and had low toxicity. Additionally, QDs have been utilized to measure cell motility via imaging of phagokinetic tracks. It has been shown that cells have been able to engulf the QDs, through a mechanism that was undefined(13,77,78).

In Vivo applications of Quantum Dots

Many research groups have focused their research on the use of QDs in vivo, replacing the fluorescent polymers and the organic dyes. QDs have superior qualities over the conventional fluorescent dyes, as QDs are photostable when used during long-term experiments. Akerman et al. have applied specific targeting of peptide-QDs bioconjugates in mice. QDs have been conjugated to peptides that specifically target lung blood vessel endothelial cells, tumor cell blood vessels, and tumor cell lymphatic vessels were conjugated to QDs, the QD-bioconjugate were introduced intravenously into mice. QD

bioconjugates have specifically targeted the lung and tumor vasculature, with no observed toxicity (74). Also, it has been noticed that the QDs have accumulated in the liver, Spleen and the targeted tissues. On the other hand, when the quantum dot conjugates were specifically targetting tumor, the QD-bioconjugates have only accumulated in the tumor cells, which indicates that the QD-bioconjugates were able to specifically target the tumor cells.

Moreover, QDs have displayed great promise when imaging the vascular networks of mammals such as lymphatic and cardiovascular systems.It have been demonstrated by Kim et al. that the NIR fluorescence of QDs could be used to locate the position of sentinel lymph nodes in mice and pigs(79). Various studies have focused on the movement of QDs through the lymph system to nodes, this application have been of tremendous importance in assisting surgeons to locate and remove lymph nodes in special medical cases (80,81).

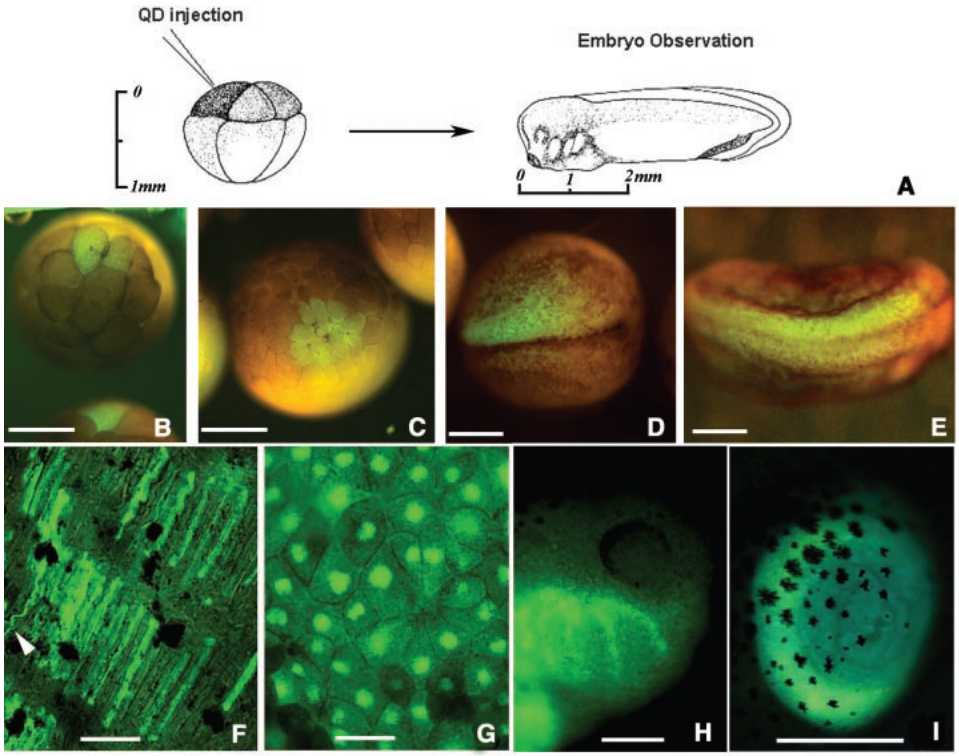

FIGURE 14.8

QDs labeling of *Xenopus* embryos at different stages and specific QD intracellular localizations. (**A**) Schematic showing the experimental strategy. QD-micelles, were injected into an individual blastomere during very early cleavage stages (B) to (E), transmission and fluorescence images have been superposed. (**B**) Injection of one cell out of an eight-cell-stage embryo resulted in labeling of individual blastomeres. (**C**) Same embryo shown 1 hour later. The daughter cells of the injected blastomere are labeled (**D**) and at a later stage (**E**) show two neurula embryos, which were injected into a single cell at the eight-cell-stage in the animal pole. The QDs can be visualized through the pigmented layer of the epidermis. (**F**) Intracellular labeling of an axon (arrow) and somites at tadpole stage 40. The QD-micelles migrate into axons all the way to growth cones. In the somites, the QD-micelle seems to localize in subcellular structures. (**G**) QDs localized in the nucleus during mid-blastula stages. This localization is reduced in later stages of the development. (**H**) Labeled neural crest cells migrating into the branchial arches. (**I**) QD fluorescence observed in the gut of an injected embryo. Printed from (41) with permission

Gold nanoparticles for intracellular imaging

Gold nanoparticles (GNPs) have been used in several biological applications; like drug delivery of hydrophobic drugs, photothermal agents, radiotherapy dose enhancer and in nanosensing. Moreover, recent studies have shown that gold nanoparticles are promising candidates for cancer stem cell therapy. Ai et al. have utilized gold nanoparticles in cancer cell imaging and photodynamic therapy (PDT). The GNPs have been functionalized with AS1411 aptamer and with one prphyrin derivative. Cancer cells (HeLa cells) have been targeted due to their overexpressed nucleolin. The functionalized GNPs were able to specifically bind to the cell surface through specific interaction between the AS1411-nucleolin and the AS1411 aptamer conjugated to the GNP. Hence the conjugated GNPs were able to differentiate between the cancer cells and the normal cells. The cancer cells were distinguished by the increase in their fluorescence intensity, caused by the specific binding of the GNPs(82).
Also, Kim et al. have applied GNPs for gene delivery(83). The GNPs were covalentely bound to small interfering RNA to its surface. siRNA-gold nanoparticles were introduced onto PBMCs or 293T cells. This study has found that the covalentely conjugated siRNA-gold nanoparticles were highly stable. The amount of GNPs uptake into the cells, have increased with increasing the concentration of the functionalized GNPs, in a linear manner. The GNPs internalized by the cell have been located in membrane-bound organelles with electron-dense cores, and they were not free in the cytosol. Also, the oligonucleootides bound to the GNPs have activated the immune-related genes and pathways in human peripheral blood mononuclear cells.

Encoding

Single coulour QDs could be applied to color-code or identify objects. Also, differently couloured QDs can produce clear spectral codes that can be used effectively in multiplexed assays. QDs are highly advantageous when used to produce spectral codes, because different colours of QDs can be excited at a single excitation wavelength and they are photo-resistant. The color -coded units can be successfully decoded using imaging techniques to determine their unique fluorescence spectra(84,85).
In the same vein, Han et al. have applied the multicolor coding in biological assays. Various sizes of CdSe/ZnS QDs have been embedded into polymer microbeads(85). The unique optical properties of QDs made them suitable for wavelength-and-intensity multiplexing. In this study, six colors of QDs having ten different intensity levels. It have been found that the QD-embedded beads were highly stable and uniform, also it has been shown that DNA hybridization studies show that the coding and target signals could be read at the single bead level.

Flouroimmunoassays using antibody-conjugated Quantum dots

Quantum dots are highly stable, with excellent quantum yields that can reach up to 90%. The relatively intense luminescence of QDs could be detected at concentrations comparable to standard fluorescent organic dyes (86). Many conjugation protocols have been developed to conjugate quantum dots to antibodies. One of the main applications of Antibody-conjugated QDs is their use in fluoroimmunoassays for the detection of proteins or small molecules. Goldman et al. have developed a conjugation strategy based on electrostatic self-assembly between negatively charged dihydrolipoic acid (DHLA)-capped CdSe/ZnS QDs and positively charged proteins, that will form a bridge between the quantum dot and the antibody(87).

Wang et al. have synthesized CdTe QD- bioconjugates based on antibody−antigen interactions. In this case bovine serum albumin (BSA) was the antigen conjugated to red fluorescent CdTe QDs. On the other hand, Anti-BSA antibody (IgG) has been coupled to green fluorescent CdTe QDs. The bioconjugates have been characterized by SDS−PAGE electrophoresis, gel-permeation HPLC, and circular dichroism. The Antigen−antibody binding affinity was assessed by enzyme-linked immunosorbent assay (ELISA). Additionally, FRET analysis have shown that the luminescence of green-emitting NPs have been quenched whereas the emission of the red-emitting NPs have been enhanced. However, the luminescence of the two colored CdTE QDs has been recovered as the immunocomplex was exposed to an unlabeled antigen(88).

Conclusions

This chapter has demonstrated the tremendous applications of nanotechnology in biological applications. More specifically, we have shed the light on the use of semiconductor nanoparticles in biology. The superior set of properties that quantum dots offer makes them suitable alternatives of the traditional organic dyes. Also, we have shown many examples on promising biological applications of QDs. QDs have made the single-molecule detection feasible. Plus, the use of QDs in encoding has great potential to reform high-throughput biology. The spectral coding technology is anticipated to open new eras in gene expression studies, high-throughput screening, and medical diagnostics. Future research will explore the use of the multiple emission colors of quantum dots for tracking of intracellular compartement.

Additionally, the toxicity of nanomaterials is a major factor that scientists should focus on during their research. Many research groups have already paid so much attention on the toxicity of QDs, some of them have discontinued the use of Cd containing QDs, and replaced them with less toxic alternatives such as; Indium, Silicon…etc. When toxicity is observed, it has been found to occur from controllable parameters, such as NP stability or dose. Hence, it is doubtful that toxicity issues should hinder the development of the exciting bio-applications offered in this chapter.
In conclusion, the surface functionalization of nanocrystals adds more functions and uses for the nanoparticles, opening doors to new biological applications. Advances involving the surface modification of QDs have enabled many novel biological experiments. Albeit, quantum dots could be challenging to deal with in every application, it is very likely that they will become a leading fluorescent probe in biology over the next several years.

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