

A review on Quantum Dots (QDs) and their biomedical applications

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Abstract – Quantum dots (QDs) are nanoscale semiconductor crystals that possess special characteristics, and they are used in various fields. The crystals are composed of elements that usually lie within the groups II–VI or III–V respectively. The diameter of these crystals is usually smaller than the Bohr excitation radius. These crystals have unique photochemical and photo-physical properties. There are several methods for synthesizing QDs, in which the high temperature co-ordinated solvents-based synthesis is one of the most important. The various synthesis procedures affect the size of these QDs, which determine their characteristics and consequently their applications. QDs are widely used in the fields of bio-imaging, photovoltaic, catalysis, light-emitting diodes, photoconductors, and photodetectors, respectively. The major factors which influence the use of QDs in the field of bio-imaging are their high luminescence, and narrow emission properties. In this review, we discuss various methods of QDs synthesis and their applications in different fields, specifically in today's world of modern biology.

Keywords: Nanocrystals, QDs, Imaging, Biomedical applications

Introduction

Quantum dots are inorganic spherical nanocrystals usually ranging from 1 to 10 nm. The crystals are composed of elements from the group II–VI or III–V, having a radius smaller than the Bohr radius respectively [1]. In 1980, Russian Physicist Alexei Ekimov of the State Optics Institute Vavilov (Leningrad) was successful in synthesizing nanocrystals of copper chloride and cadmium selenide and observed fluorescence and a gradient of colours. Later on, these observations were published. Just after a few years, Russian Physicist Alexander Efros published a report where he tried to explain the behaviour of these nano-sized crystals. Inspired by this report, Louis Brus, later successfully produced nanocrystals which were in liquid form (CuCl dispersed in a transparent insulating matrix) and thus published in 1983 [2]. Quantum dots (QDs) have excellent optical and electronic properties which again are influenced by their size. Quantum dots also are known to display unique electronic properties which are intermediate between bulk semiconductors and discrete molecules [3].

Over the past decades, there has been extensive research on these particles and their various applications. These have semiconductor-like properties which vary in size. The particle size of these nanocrystals largely determines many of their properties, most importantly the wavelength

of fluorescence emission. The light produced by QDs nanocrystals is much more saturated and also their colour depends on the size of the nanoparticles, which are seen to vary from 2 nm to 10 nm. The sizes of the QDs are largely dependent on the way of synthesis. It is reported that QDs size increases as their reaction time increases. Due to their unique properties, QDs are widely used in many fields. In the field of biology, they are used in fluorescence resonance energy transfer (FRET), gene technology, fluorescent labelling, cellular tracking, detection, and imaging, and even in the field of tumours and cancers. Apart from biology, QDs are also widely used in the field of photo-catalysts, electronics, pharmaceuticals, bio sensing, etc.

Types of quantum dots

Commonly, typical QDs consist of an II–IV, IV–VI, or III–V semiconductor core (e.g., CdTe, CdSe, PbSe, GaAs, GaN, InP, and InAs) which again are surrounded by a semiconductor shell having a wide bandgap, i.e. ZnS. QDs may be of heavy metals or silicon-based and each has its specific properties.

Quantum dots may be majorly classified into two major types based on their composition and structure, respectively.

- (i) Core type quantum dots (Core shell quantum dots)
- (ii) Alloyed quantum dots

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i. Core type quantum dots

This type of nanocrystals is generally single component materials with a uniform internal composition, i.e. chalcogenides (which include selenides, sulfides, or tellurides) of metals like cadmium, lead, or zinc. These particles exhibit luminescence which again arises from their electron-hole pairs recombination. They consist of a single material, mainly metal chalcogenides). The core-shell semiconductors nanocrystals usually consist of a core-type material encapsulated within another second semiconductor material having a higher band gap.

ii. Alloyed quantum dots

Alloyed quantum dots are made up of multiple materials, but in a homogeneous mixture rather than distinct regions separately. The combination of two different semiconductors with different band gaps imparts new properties, distinct properties to the particles that are distinct from the original materials, thereby making the material more effective especially improving the optical and electronic properties. Thus, they possess novel and additional compositions apart from their general ones.

Properties

1) Structure of quantum dots

The core structure of QDs has many numbers of atoms that lie between the atomic-molecular level and bulk material having a band gap which again is dependent on factors such as bond type and strength with the nearest neighbours. In the case of isolated atoms, there are observed sharp and narrow luminescent emission peaks, but nanoparticles made up of approximately 1000 atoms show distinct narrow optical line spectra, which is why QDs are often described as artificial atoms (δ -function-like DOS) [4]. QDs require sufficient control during their synthesis which alters their properties, as their intrinsic properties are determined by different factors, such as size, shape, defect, impurities, and crystallinity. This dependence on size arises from changes in the surface-to-volume ratio with size and quantum confinement effects. Nevertheless, QDs also exhibit different colours of emission with changes in size.

2) Surface structure

QDs have excellent optical characteristics, which again are influenced by their surface-to-volume ratios.

This high surface-to-volume ratio is seen to allow an enhanced or reduced transfer rate of photo-generated charge carriers due to the high density of surface sites. These surface states of the QDs are seen to influence optical absorption (photoluminescence excitation – PLE), quantum efficiency, luminescent intensity, and spectrum and aging effects [5].

Generally, surface states arise from unsatisfied bonds at the reconstructed surfaces and are seen to be affected by

non-stoichiometry and voids. Thus, energies of these surface states generally lie in the bandgap of the QDs respectively [6]. Hence, they can trap charge carriers (i.e., electron or hole) and behave as reducing (electron) or oxidizing (hole) agents. It had been observed that these electrochemical reactions or behaviour at the surface significantly can affect the overall conductivity and optical properties of QDs, because of which, the surface states have significant effects on the optical and optoelectronic properties of the QDs. Surface passivation of QDs can confine the carrier inside the core and improves the optical properties of QDs, but this passivation layer acts as either insulator or barrier for the conduction of charges.

3) Surface passiveness

The surface defects in QDs act as temporary “traps” for the electron, hole, or excitons, quenching radiative recombination and reducing quantum yields (QC). Hence, capping or passivation of the surface is vital for the development of photo-stable QDs [48–50].

Principally, a perfectly passivated surface of a QD has bonds saturated, and, hence, exhibits no surface state, and the near band-edge states are quantum-confined internally. In the case of a compound semiconductor, if the anion dangling bonds at the surface are not passivated, a band of surface states is expected in the gap just above the valence band-edge. Hence, surface modification of QDs is very demanding and is generally carried out by depositing an organic or inorganic capping layer on the QDs, respectively.

Synthesis of quantum dots

Synthetic approaches for QDs majorly comprises of two processes: Top-down and Bottom-up approaches. Figures 1 and 2 summarize the major synthetic methods for different nanoparticles and the top down and bottom up approaches of nanoparticle synthesis, respectively.

1) Top-down approach

In this approach, a bulk semiconductor is thinned leading to the formation of QDs (Fig. 2). Several techniques such as electron beam lithography, reactive-ion etching, and/or wet chemical etching are commonly used to achieve QDs of diameter ~ 30 nm [44]. The controlled shapes and sizes along with the desired packing geometries are achievable by the quantum confinement effect. Parallely focused ion or laser beams have also been used to fabricate arrays of zero-dimension dots. The major drawbacks of these processes mainly include the incorporation of impurities inside the QDs, leading to structural imperfections in the QDs [7].

An old technique called Etching, known for more than 20 years, plays a crucial role in the nanofabrication processes. In the process of dry etching, reactive gas species are inserted inside a chamber to which a radio-frequency voltage is applied, thereby leading to the formation of plasma which breaks down further the gas molecules into reactive fragments, respectively [51].

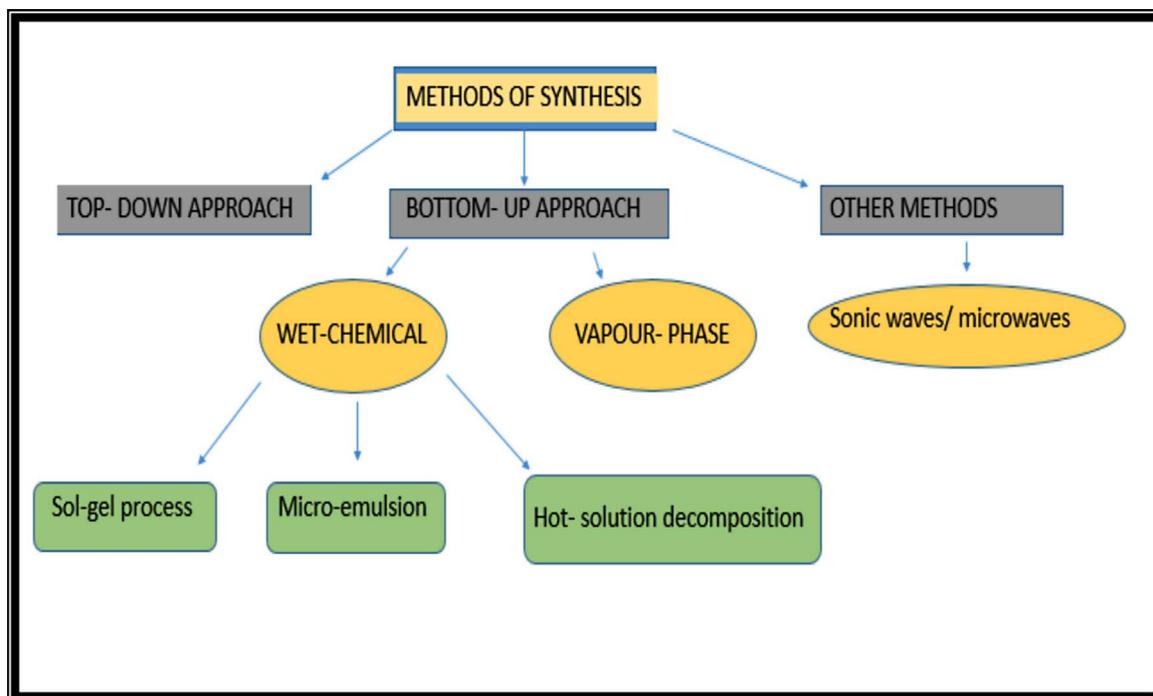


Figure 1. Various methods of synthesis of QDs (the synthesis method affects their sizes and various properties).

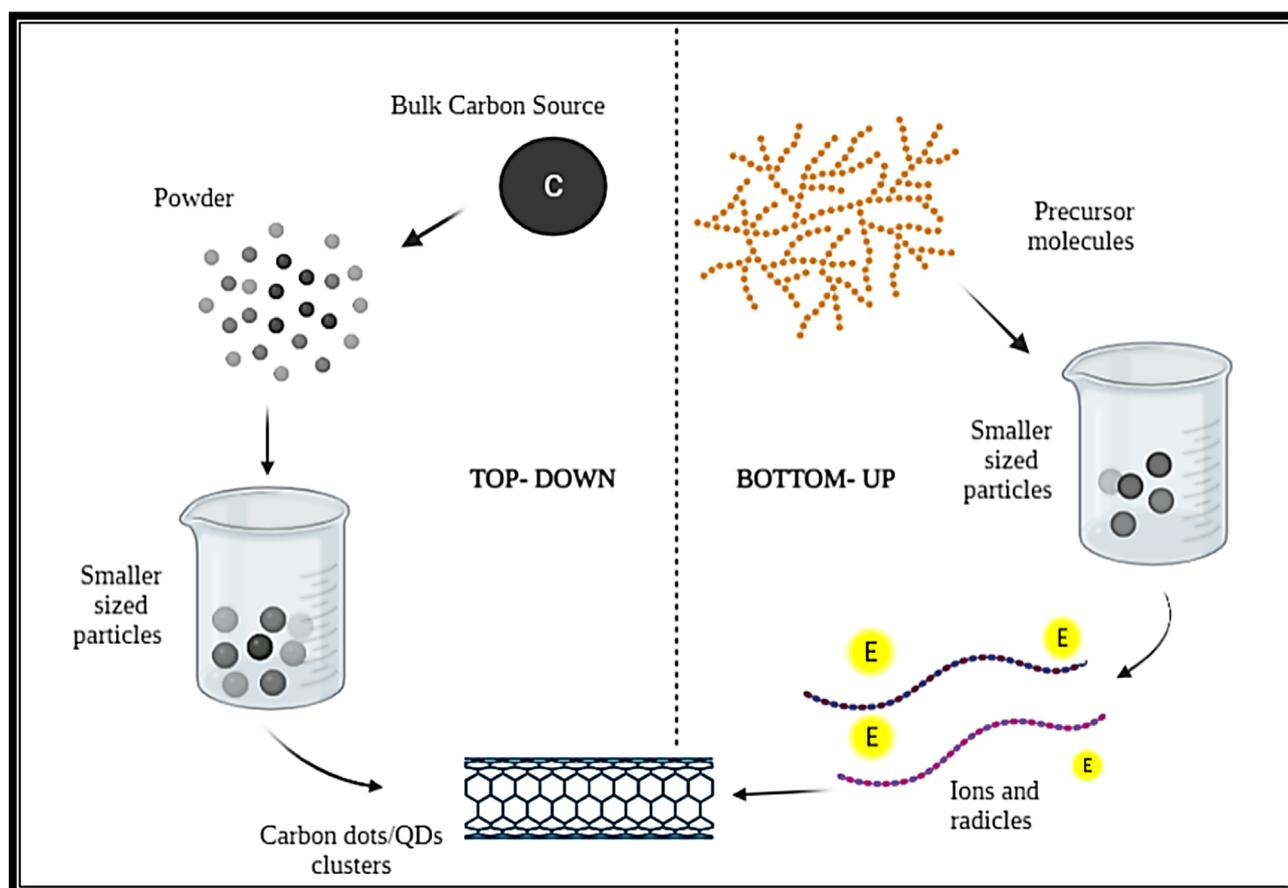


Figure 2. Top-down and bottom-up approach schematic.

These high kinetic energy species strike the surface, thereby forming a volatile reaction product to etch a patterned sample. Now when the energetic species are ions, this etching process is called reactive ion etching (RIE). These RIE processes have been used to produce close-packed arrays for testing of lasing in QD semiconductors. Apart from this, there exists another method to achieve patterns with QDs dimensions, which is the use of electron beam lithography followed by etching or lift-off processes. This method offers a high degree of flexibility in the design of nanostructures systems as reported. This method was employed for the synthesis of III–V and II–VI QDs with particle sizes as small as 30 nm successfully [8, 45].

2) Bottom-up approach

There have been reported several different self-assembly techniques which have been used to synthesize QDs and can be further subdivided into wet-chemical and vapour-phase methods, respectively. Under wet-chemical methods, techniques such as micro-emulsion, sol–gel, competitive reaction chemistry, hot-solution decomposition, and electrochemistry. Under vapour-phase techniques such as molecular beam epitaxy (MBE), sputtering, liquid metal ion sources, or aggregation of gaseous monomers all fall under this category, respectively [7, 9] (Fig. 2).

2.1) Wet-chemical methods

This technique mainly follows conventional precipitation methods with careful control of parameters for a single solution or a mixture of solutions. This precipitation process invariably involves both nucleation and limited growth of nanoparticles. The nucleation may be categorized further as homogeneous, heterogeneous or secondary nucleation. In Homogeneous nucleation, the solute atoms or molecules are seen to combine and reach a critical size without the assistance of a pre-existing solid interface [7].

Varying factors, such as temperature, electrostatic double layer thickness, stabilizers or micelle formation, concentrations of precursors, ratios of anionic to cationic species and solvent, helps in achieving QDs of the desired size, shape and composition, respectively [10].

2.2) Sol–gel processes

Sol–gel techniques have been used for many years to synthesize nanoparticles including QDs. In this typical technique, a sol (nanoparticles dispersed in a solvent by Brownian motion) is prepared using a metal precursor (i.e., alkoxides, acetates, or even nitrates) in an acidic or basic medium. There exist three main steps in this process; these are hydrolysis, condensation (sol formation), and growth (gel formation) [61, 63].

Generally, the metal precursor hydrolyzes in the medium and condenses to form a sol, following which polymerization occurs to form a network (gel). This method has been used to synthesize II–VI & IV–VI QDs, such as CdS, ZnO, and PbS QDs as reported. For example, ZnO QDs have been prepared by mixing solutions of Zn-acetate in

alcohol and sodium hydroxide, followed by controlling aging in the air. The major advantage of this process is simple, cost-effective, and suitable for scale-up. The major disadvantages of this sol–gel process include a broad size distribution and a high concentration of defects. Hence, this synthesis technique is used sparingly [11, 46, 47].

2.3) Micro-emulsion process (reverse micelle technique)

The micro-emulsion processes are popular methods used for synthesizing QDs at room temperature. The overall processes can be categorized as either normal micro-emulsions, i.e., oil-in-water, or as reverse micro-emulsions, i.e., water-in-oil respectively. It is seen that in some cases, other polar solvents, e.g., alcohol, may be used instead of water as well.

Among the two, the reverse micelle process is popular for synthesizing QDs, in which the two immiscible liquids (polar water and nonpolar long-chain alkane) are mixed and stirred to form an emulsion [12].

As reported the reverse micelle technique has been used to prepare II–VI core and core/shell QDs, such as CdS, CdS:Mn/ZnS, ZnS/CdSe, CdSe/ZnSe, ZnSe and IV–VI QDs. The advantages of this process are easy control of the QDs size by changing the molar ratio of water to surfactant; a narrow distribution of size as compared to the sol gel process, and ease of dispersion of the QDs. Disadvantages mainly include low yield and incorporation of impurities and defects, respectively [13].

2.4) Hot-solution decomposition process

In 1993, Bawendi and associates established a warm-temperature pyrolysis technique for the assembly of QDs. during this Precursors, like alkyl, acetate, carbonate, and oxides of Group II elements, are mixed with Group VI phosphine or bis(trimethylsilyl) precursors respectively [13]. In this procedure, degassing followed by drying of trioctyl-phosphine oxide (TOPO, a coordinating solvent) at 200–350 °C under vacuum was tired in a three-neck round flask within a dry box. As reported, a combination of Cd-precursor and tri-n-octyl-phosphine (TOP) selenide was prepared within the dry box and further injected together with vigorous stirring into the flask at a temperature of ~300 °C. This simultaneous injection of precursors into the flask together with TOPO resulted in homogeneous nucleation which again further led to the formation of QDs. The Ostwald ripening method was utilised, which led to the growth of enormous nanoparticles at the expense of smaller ones via the formation and decomposition of intermediate chemical species. In this technique (Ostwald ripening), the upper free energy of smaller QDs makes them lose mass to large-size QDs, thus eventually disappearing. This leads to a slow increase of the scale of QDs at a temperature of 230–250 °C, which again depends on precursor, coordinating agents, and solvents. This leads to a slow increase in the production scale of QDs at temperatures between 230 and 250 °C. This whole process however depends on precursors, coordinating agents, and solvents respectively. This coordinating TOPO solvent then stabilizes the QDs dispersion, improves the passivation of the

surface, and provides an adsorption barrier to slow the whole process. Eventually, the QD's size is principally controlled by the overall reaction and temperature. The new solution method, as reported, is extensively accustomed to synthesize II–VI, IV–VI, and, III–V QDs. The distinctive advantage of this process is that it provides sufficient thermal energy to anneal defects and ends up in mono dispersed QDs. Now, since the expansion of the particles during this process is comparatively slow and may be controlled by modulating the temperature, a series of QDs sizes is prepared from the identical precursor bath. Thus, by using these processes, large quantities of QDs are produced over the years. The disadvantages of this method include higher costs thanks to the employment of extreme temperatures, the toxicity of some organo-metallic precursors, and usually poor dispersions in water [14, 15].

2.5) Vapour-phase methods

The vapour phase method for producing QDs generally begins with processes during which layers are grown in an atom-by-atom process. Within the early stages in place, self-assembled nano-structured materials were produced by hetero-epitaxial growth of highly strained materials. Techniques like, Molecular beam epitaxy (MBE) are reported to be accustomed, deposit these over layers and grow elemental, compound, or alloy semiconductor nano-structured materials on a heated substrate under ultra-high vacuum ($\sim 10^{-10}$ Torr or 7.5×10^{-16} Pa) conditions generally. The essential concept behind MBE process is evaporation from an open source to make a beam of atoms or molecules. This beam within the MBE technique is created from solids (elements i.e., Ga and As) to provide GaAs QDs, or a mix of solid and gases. It has been seen that metal-organic sources may leave high concentrations of carbon within the QDs. This method has been mainly used for self- assembling the QDs from III–V semiconductors and II–VI semiconductors. Techniques like beam heating, resistive or joule heating, arc-discharge and pulsed laser ablation are accustomed to cause evaporation [16]. Factors like strain and surface energies mainly control the formation of QDs majorly from the deposited thin films [17, 64].

3) Other methods

There exists other methods for producing QDs, among these are sonic waves and micro-waves.

Ultrasound waves have been seen to be involved within the synthesis of QDs, within the size of 1–5 nm by formation, growth and implosive collapse of bubbles within a liquid colloidal solution which was used for the synthesis process [18]. These sounds lead to the generation of localized hotspot through adiabatic compression within the gas inside the collapsing bubble, thereby enabling the reactions that form QDs. In this approach, acetate precursors of metal ions were diffused during a solution to which seleno-urea was added for about an hour under argon atmosphere. For the assembly of QDs the temperature of the solution mixture rose to 80 °C. Quite similar processes like hydrothermal

synthesis methods are quite successful in producing QDs of desired sizes. These processes involve crystallization of inorganic salts from solution which again are controlled by pressure and temperature. The solubility of those inorganic compounds generally lowers because the temperature and/or pressure is lowered, thus resulting in crystalline precipitation formations. By altering the pressure, temperature, reaction and aging time and reactants varying sizes and shapes of QDs were achieved successfully (Tab. 1).

Biological applications of quantum dots

The numerous uses of quantum dots may be categorised under the following key headings:

1. As Cellular probes [4].
2. Fluorescence resonance energy transfer (FRET) [19].
3. Cell tracking and intracellular delivery (Immuno-labelling) [34].
4. Radio opacity and paramagnetic properties.
5. MRI contrast agent, it has been shown that QDs can be used for bio-imaging purposes [20].
6. Tissue staining.
7. Gene delivery [52, 53].
8. Cellular imaging [21, 22].
9. Cellular motility assays [54, 55].

1) Imaging/Bio-imaging

An interesting application of QD is in the field of biology, where imaging techniques such as magnetic resonance imaging (MRI), optical imaging, and nuclear imaging are in high demand. Each technique differs from each other in terms of sensitivity, resolution, complexity, acquisition time, and operational cost and is complementary to each other as well. Presently, research is aimed towards the unique properties of QDs and their use in biological imaging. Traditional dyes are mainly used for optical bio-imaging despite having several drawbacks associated with their use. Normally, cells auto-fluorescence in the visible spectrum and lead to several effects. Due to this, auto-fluorescence, there occurs signal masking from labelled organic dye molecules, there occurs instability of organic dye under photo-irradiation is well known in bio imaging which results in only short observation times, also, the general conventional dyes have a narrow excitation window which makes simultaneous excitation of multiple dyes difficult, the dyes are sensitive to pH variations and most importantly, the organic dyes have a broad emission spectrum with a long tail at red wavelengths which creates spectral cross talk between different detection channels and makes it difficult to quantify the amounts of different probes [23].

Now quantum dots, on the other hand, which are of interest in biology, have many striking features. These include: higher extinction coefficients; less photo-bleaching; absorbance, and emissions can be tuned with size; generally broad excitation windows but narrow emission peaks; multiple QDs can be used in the same assay with minimal

Table 1. Examples of synthesized quantum dots with their precursors, sizes and their respective synthesis processes.

Quantum dot	Synthesis process	Size (nm)	Medium precursor	Application	References
CdSe	Bottom-up approach (hot-solution decomposition process)	~6 nm	Me ₂ Cd, Se, TBP, TOPO, HPA	FRET	Peng et al. [32]
CdSe/ZnS	Bottom-up approach (hot-solution decomposition process)	4.5–5 nm	Me ₂ Cd, Se, TOP, TOPO, HDA, (TMS) ₂ S, Me ₂ Zn	FRET	Manna et al. [33] Hikmet et al. [29]
PbS	Bottom-up approach (hot-solution decomposition process)	5 nm	PbO, OA, (TMS) ₂ S	Photo-electronic devices	Bakueva et al. [35]
ZnSe/ZnS	Vapour-phase method	1–1.9 nm	TOPO, diethyl zinc, L hexa-methyl-disilathiane, TBP	FRET, LEDs	Kim et al. [36, 65]
CdSe/ZnSe/ZnS	Hot solvent mixture (bottom-up approach)	~8.6 nm	TOPO, diethyl zinc, L hexa-methyldisilathiane, TBP, CdO:Se molar ratio of 1:5	Intra-cellular pH sensors	Liu et al. [37]
C-QDs (Carbon quantum dots)	Hydrothermal (green material used as source)	4–6 nm	Banana peel waste	Bio imaging	Atchudan et al. [38]
C-QDs (COC dots), sulfur doped with hydrophilic groups at the surface	Ultrasonication (other methods)	1–4 nm	Waste chimney oil	Sensors, bio-labeling, and ink	Das et al. [39]
C-QDs (Carbon quantum dots)	Pyrolysis (heat synthesis)	6 nm	Finger-milletragi, CaCl ₂ ·2H ₂ O, CuSO ₄ ·5H ₂ O, Ni(NO ₃) ₂ ·6H ₂ O, Mn(NO ₃) ₂ ·6H ₂ O, MgCl ₂ ·6H ₂ O	Biosensor	Murugan et al. [40, 60]
Graphene QDs (colloidal)	Modified hummers method (heat and ultrasonic treatment)	2.9–3.6 nm	Graphene oxide sheets	HeLa cell line Cell nucleus (bio-imaging)/ cellular imaging	Pan et al. [41, 62]
Boron-doped graphene quantum dots	Hydrothermal process (modified hummers process)	3–7 nm	Graphite rods, 0.1 M borax	Stem-cell imaging (bio-imaging), cellular imaging	Fan et al. [42, 43]

interference with each other; toxicity may be less than conventional organic dyes, and the QDs may be functionalized with different bio-active agents. Normally monochromatic light is used for intra-vital microscopy, but in-order to image deeper tissues the technique which is required is NIR (Near infrared spectroscopy) light. It has been reported that these NIR-emitting QDs have been used to avoid interference from the auto-fluorescence. By nature, inorganic QDs are more photo-stable compared to organic molecules when excited by ultraviolet rays; also, their fluorescence is more saturated [24, 25, 56–59].

Researchers have used QDs for in vivo and in vitro imaging and diagnostic of live cells as a complement to or replacement for conventional organic dyes, respectively.

1.1. Applications in in-vitro cellular imaging

Certain classes of QDs are used in in vitro imaging to label cells. For example, CdTe capped with 3-mercaptopropionic acid (3-MPA) were used as an imaging tool to label

Salmonella typhimurium cells. Also, Jaiswal et al. reported labelling *HeLa* cells with acid capped CdSe/ZnS QDs. Scientists such as Gac et al. have successfully detected apoptotic cells by conjugating QDs with biotinylated Annexin V, which enabled the functionalized QDs to bind to phosphatidylserine (PS) moieties present on the membrane of apoptotic cells but not on healthy or necrotic cells. With this detection and imaging of apoptotic cells, monitoring specific photo-stable apoptosis was possible. Reports published by Wolcott, suggested that when QDs, which were silica-coated, CdTe were decorated with functionalized groups, it was not only used for labelling but also was seen to prevent toxic Cd²⁺ leaking from the cores. Recently, biocompatible, and nontoxic QDs were made from rare earth (RE) elements such as Gd-doped ZnO. This rare earth elements-based QDs are seen to provide distinct advantages over traditional heavy metal-containing QDs. Over the recent years there has been a demand over MRI-detectable QDs [23]. QDs have also been used in several cancer studies. Majorly, these QDs are used in the

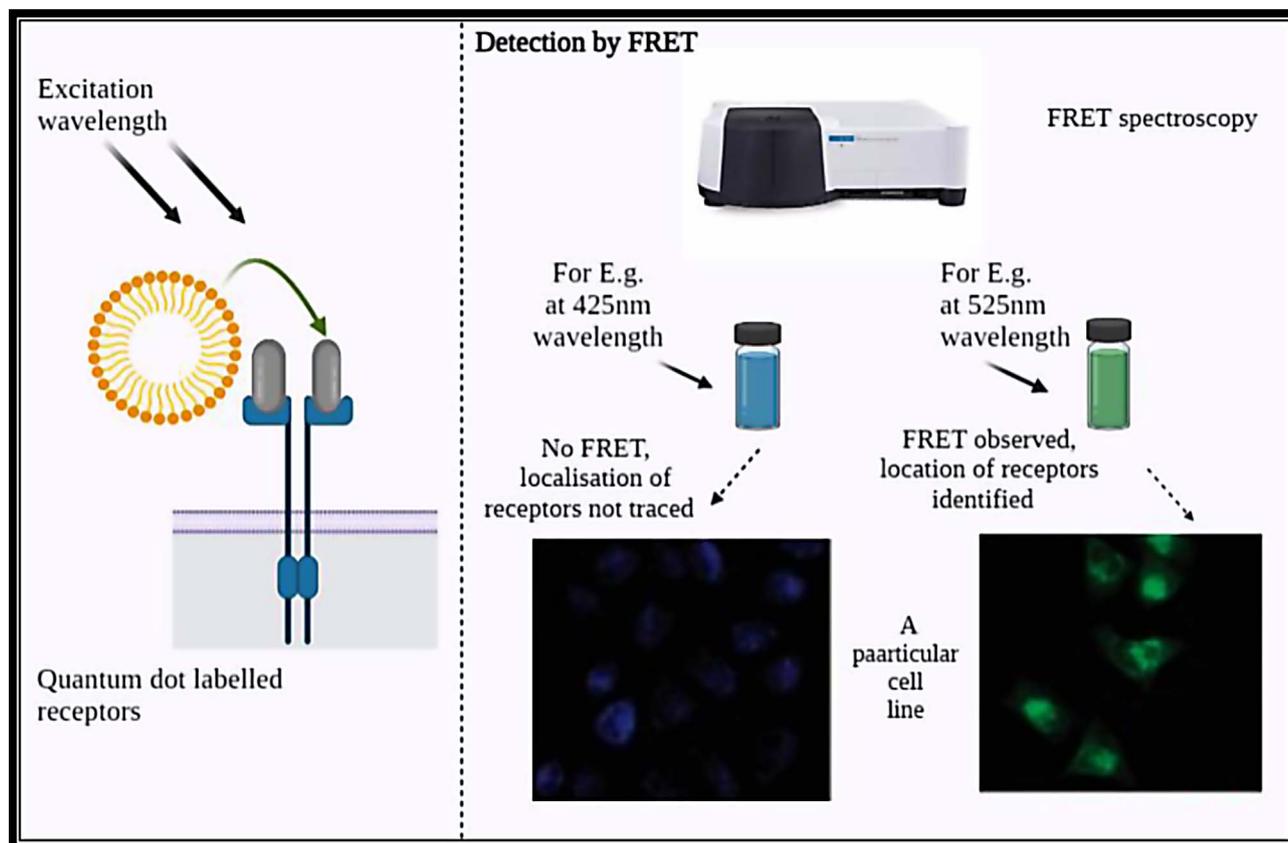


Figure 3. Quantum dots and their application in FRET imaging.

tracking and diagnosis of cancer cells. Cao et al. have also reported the use of near-IR (650–900 nm) QDs, having an emission wavelength of 800 nm (QD705) to label squamous cell carcinoma cell line U87 (U87/QD705) [26]. Apart from this, another major role played by QDs are in transfection. Studies have reported that QDs mainly help in fluorescence imaging and nucleus targeting of living cells by transfection and RNA delivery [69, 73].

2) Applications in Fluorescence resonance energy transfer

QDs have multiple applications in imaging (Fig. 3). Many researchers prefer these particles over traditional organic dyes because of their unique and well-defined properties. Two major advantages that make these quantum particles be preferred over traditional organic fluorophores for FRET are:

1. These particles can be size tuned to overlap with a particular acceptor dye's absorption.
2. These quantum particles have several acceptor dyes which interact with a single QD-donor particle, thereby substantially improving the FRET efficiency successfully [19].

FRET generally involves the transfer of fluorescence energy from a donor particle to an acceptor particle whenever the distance between the donor and the acceptor is smaller than a critical radius, respectively. FRET is a

technique used which is sensitive to molecular rearrangements. Researchers have long used this photo-physical process (FRET) to monitor intracellular interactions and binding events within the cell. In Figure 3 an illustration of this activity has been displayed. The conformational change observed during FRET has been used in measurements of proteins and has been used in monitoring protein interactions and assaying of enzyme activity, respectively. Over the years, several groups of scientists have attempted to use QDs in FRET technologies; these include monitoring protein interactions in the Holliday Junction. Apart from this, QD-FRET has also been exploited for imaging activity of enzyme proteases as well, where a QD-probe conjugate is bound to a quencher probe by a peptide sequence which is recognized by a protease, in which state the fluorophore is quenched, upon cleavage of the two molecules by a protease, emission is restored, and thus the activity can be visualized [20]. Upon comparison with previous organic fluorophores-based results, QDs showed a significant increase in luminescence after incubation with a collagenase. Further studies have also showed that QD-FRET being involved in the detection activity of caspase-1, thrombin and chymotrypsin, trypsin, and b-lactamase as well. In the field of cancer as well, a QD-FRET assay of collagenase was seen to distinguish between normal and cancerous breast cells. Despite their immense applications and advantages of QD-FRET, there still exist issues that may be disadvantageous for QD in FRET. Among them, the physical

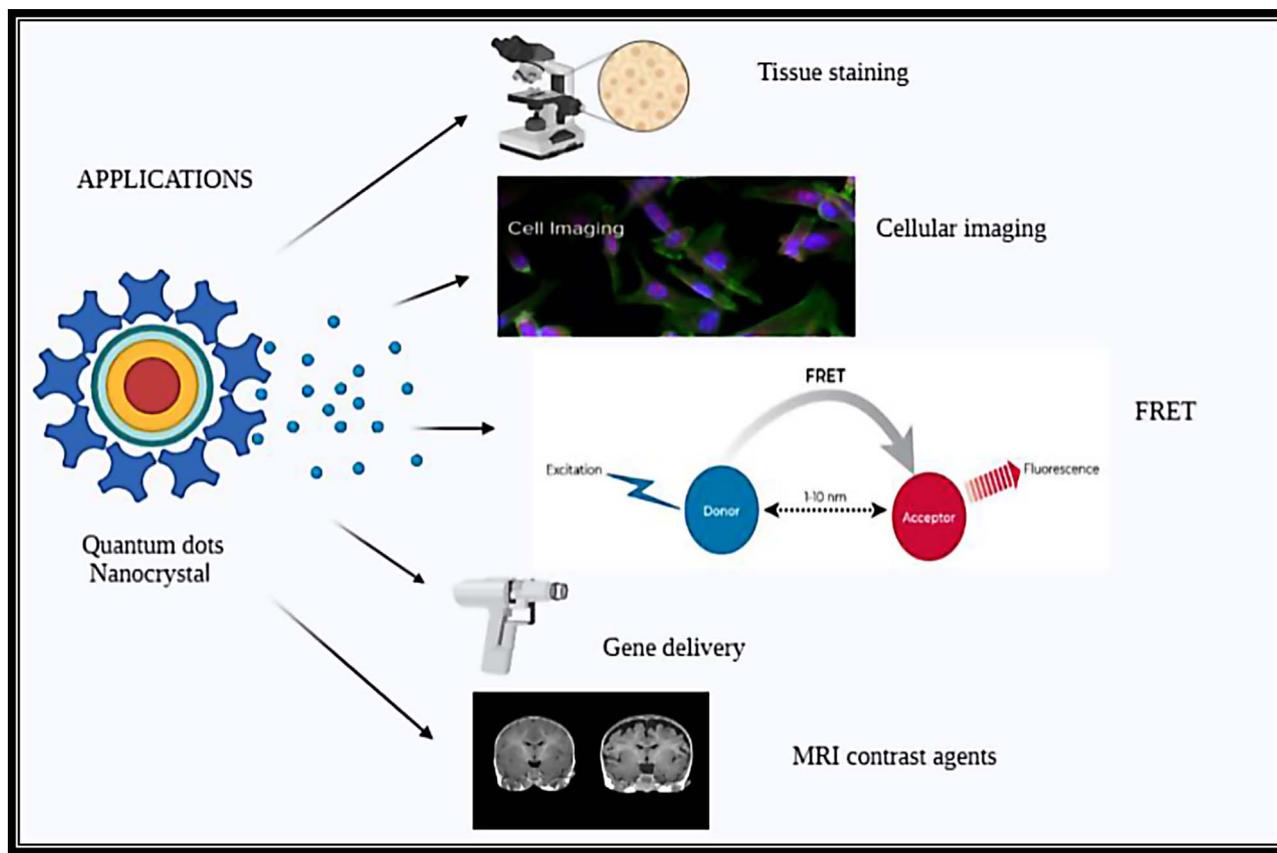


Figure 4. QDs and their applications.

dimensions of QDs, especially after capping, or the addition of shells such as DHLA (dihydrolipoic acid) make close approach to the QD core difficult, reducing FRET efficiency, though this may be partially reduced by the addition of a relay acceptor molecule. Apart from this, peptide accessibility is also a concern as to provide efficient probes. For this, multiple energy acceptors need to be conjugated to a central QD, which again results in steric hindrance for substrate accessibility by proteases [66–68, 72].

3) Other biomedical and environmental applications

Specific properties of QDs such as, high luminescence, narrow emission, low toxicity and biocompatibility enables them as a perfect candidate for bio-imaging, diagnostics, and bio-sensing applications, mostly related to biomedical and environmental sciences. Scientist demonstrated fibrous phosphorus QDs as fluorescent labels for human adenocarcinoma bio-imaging. Reports published by scientists suggest ultra-bright graphene QDs which were obtained under microwave irradiation have also been suggested for cell-imaging applications. Gerard Giraud et al. from the University of Edinburgh (UK) in his report along with a collaborative effort between the Institute for Condensed Matter and Complex Systems, the Division of Pathway Medicine, the School of Chemistry, and the National Physical Laboratory, reported the use of QD-labelling combined with fluorescence lifetime imaging microscopy for the

detection of DNA hybridization events on DNA microarrays [7, 9, 27, 28].

Raquel Ibáñez-Peral et al. from the Departments of Chemistry and Biomolecular Sciences, University and the School of Biotechnology and Biomolecular Sciences, University of New South Wales in Sydney (AU) investigated whether QDs could be used as suitable multicolour optical labels of specific nucleotide probes for microbial identification using flow cytometry (FCM). Due to the behaviour of QDs, such that their nature to fluoresce and scatter below the resolution of the flow cytometer, led the scientist to conjugate QDs to paramagnetic beads (Dynabeads). From their results they concluded that the minimum fluorophore-concentration required for detection of QDs above the auto-fluorescent background was 100-fold less than for the commonly used fluorophore FITC, even when under suboptimal excitation conditions. Even so, their research also showed that QD binding to the beads markedly influences their optical properties and this fact alone suggests further that the application of QDs for FCM needs further study and development respectively [70, 71].

4) Cell tracking and intracellular delivery

Non-invasive procedures are strongly favoured over invasive techniques in cell tracking because they avoid cell damage or change, allowing the cell to be followed in its normal condition. QDs can be used for non-invasively

labelling cells. Most approaches rely on the cells ability to endocytose. When mammalian (HeLa) cell lines and *Dictyostelium discoideum* cells were incubated with DHLA capped QDs for a period of 2–3 hr, followed by washing of the excess QDs, it was seen that in both cells QDs were present in large number of vesicles and labelled. Thus providing strong evidence that QDs were internalised by endocytosis respectively. Upon blocking endocytosis by incubating the cells at 4 °C neither of the cells was labelled even after keeping them for a period of 6 h. It was noticed that in HeLa cells, QDs labels were remarkably stable for more than a week, with no discernible consequences on cell viability [29, 30]. In another study which was independent of endocytosis, the cell surface was biotinylated with sulfo-NHS-SS-biotin, a membrane-impermeant, amino-directed coupling agent. The cells were subjected to incubation for 10 min at 4 °C with QDs and then washed to remove the unbound QDs. It was seen that the label appeared on the surface of the cell as endocytosis was not possible at 4 °C. Again, when the cells were maintained at 37 °C for a period of 2 hr, resulting in endocytosis which lead to internalization of the QDs. The findings showed that QD labelling had no negative effects on the usual endocytosis mechanism. This method also shed light on the fact that labelling can also be done on cells that do not undergo endocytosis as the QDs were present at the surface/membrane [29–31]. Figure 4 summarizes important applications of quantum dots in different biological applications.

Conclusion and prospects

QDs have undeniably provided remedies in many fields especially in the field of biology and there lies ahead more investigations which are required to be done. Due to its striking properties, and stabilities QDs are widely used in imaging purposes. Both in vitro and in vivo imaging along with FRET and many more, QDs have shown results which will help in the development of research in the coming years. In field of tumour and cancer studies QDs have also shown promising results in terms of analysis and deep tissue imaging.

Despite their utilities in many fields QDs require fine tuning especially in the case of biocompatibilities. Studies have revealed that QD nanocrystals exhibit cytotoxic and genotoxic effects and are also seen to be involved of disruptions of membranes which not only are undesirable but could also be life threatening. Nonetheless, it can thus be concluded that in the upcoming years we will be witnessing more of QDs and their uses in various industries as they are seen to have a bright future and lead us towards a more advanced lifestyle.

Conflict of interest

The authors have no conflicts of interest.

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