Quantum Dots and Biomedical Applications

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Abstract— Exploiting the properties, methods of synthesis, the possible toxicity and applications of zero dimensional nanostructure-Quantum Dot are the topics discussed in this review. An application of these QD's in biomedical research is an area of prime focus. Among the very first discoveries of nanotechnology, QD's integrated biological sciences and are widely projected to have commercial, consumer and clinical by-product.Eccentric luminescence characteristics and electronic properties like wide and continuous absorption spectra, narrow emission spectra, and high light stability are showcased by QD's. QD's because of its small structure its physical properties i.e. optical and electron transport attributes are different from those of it bulk counterparts.

Key words: Absorption spectra, Emission spectra, Luminescence characteristics, Toxicity

I. Introduction

The term "QDs" was coined by Mark Reed [1]. However, in 1981 Alexey Ekimov [3, 4] first discovered it in a glass matrix [2] proceeding to which in 1985 Louis E. Brus discovered it in colloidal solutions following which it became one of the most captivating areas of research and currently QDs are recognized as a new generation of materials. This review article introduces in chapter 2 the basics of QD's, their smart properties and concepts to explain the quantum confinement, then in chapter 3 explains the synthesis procedure of these zero dimensional quantum dots followed by giving an overview on the toxicity of quantum dots in chapter 4. The chapter 5 broadly explains the applications of QD's in biomedical domain and chapter 6 puts an end to the review article with some conclusions.

II. Definition

Technically quantum dots are defined as "small crystals containing a variable number of electrons that occupy well-defined, discrete quantum states and have electronic properties that are intermediate between bulk and discrete fundamental particle". The QD's are architecture by different types of quantum confinement as shown in the figure 1 below. On basis of these quantum confinements the QD's can be classified as:

1) Quantum dot: that confine in all three dimensions;
2) Quantum wires: that confined electrons or holes movement in two spatial dimensions and allow third dimension for free propagation;
3) Quantum wells: which confine electrons or holes in one dimension and allow free propagation in remaining two dimensions.

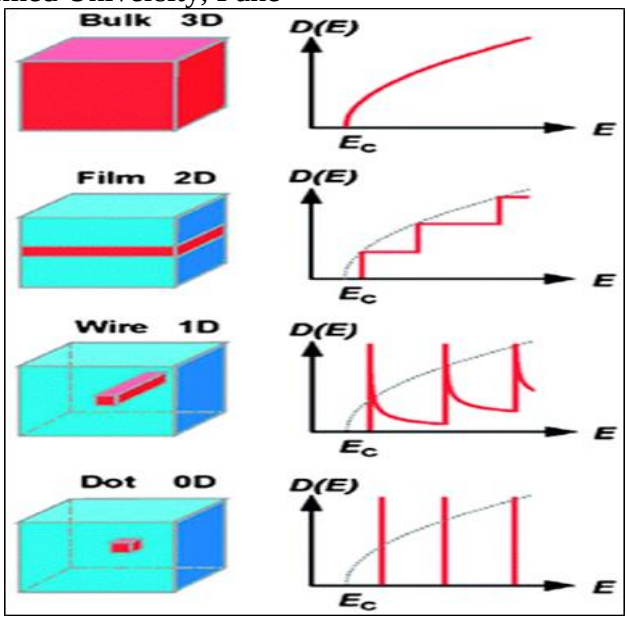

Fig. 1: Representation for different types of quantum confinement [13].

By controlling the geometrical size, shape, and confinement potential QD's are engineered. The band gap of the material determines the amount of white light the QD's will absorb reemit a specific color a few nanoseconds later. QDs being first nanotechnologies to be integrated with the biological sciences find application in number of products. For example, CdSe/ ZnS quantum dots are presently the most common commercially available product. QD range is typically between 2 and 10 nm in diameter. There is an increasing tendency to apply QDs as markers in plant science. They also have excellent photo stability and overcome the limitations associated with photo bleaching.

A. Optical Properties Of Qd's:

To overcome the limitations of current fluorophores such as organic dyes, fluorescent proteins and lanthanide chelates QD's came into picture due to its unique optical and electronic properties that were seen due its quantum confinement effect [5]. The width of the excitation spectrum, the width of the emission spectrum, photostability, and the decay lifetime are the properties that particularly influence fluorophores behaviour, and thus applicability to different situations. Conventional dyes exhibits from narrow excitation spectra which require excitation of light of a specific wavelength, which varies between particular dyes and broad emission spectra which means the spectra of different dyes may overlap to a large extent. This phenomenon limits the number of fluorescent probes used to tag different biological molecules and be spectrally resolved simultaneously. On the other hand broad absorption spectra of QD's allow excitation by a wide range of wavelengths because of which simultaneous excitation of multiple different coloured QDs using a single wavelength can be exploited. Further QDs have

narrow emission spectra, which can be controlled by variation of core size and composition, and through variation of surface coatings in a relatively simple manner. They can be engineered to emit light at a variety of precise wavelengths from ultraviolet (UV) to infrared (IR). Thus multiplexed imaging where multiple colours and intensities are combined to encode genes, proteins and small-molecule libraries [6, 11] can be carried out due to narrow emission and broad absorption spectra of QDs. Almost 5–6 colours with 6 intensity levels can be used to give a yield of approximately 10,000–40,000 different recognisable codes [9] using realistic schemes. QD's give the opportunity to track the long-term synergy of multiple-labelled biological molecules in cells as a result of its good photostability property as discussed below.

In most fluorescence applications photostability is a critical feature along with particular advantages of QDs. QD's being extremely stable are capable of going through numerous cycles of excitation for hours together with brightness and photo bleaching threshold at high levels which is not possible in organic fluorophores as they bleach for few minutes on exposure to light [6, 7]. Out of the many organic dyes available including Alexa488 [10] which is at present reported as the most stable organic dye, QDs have the ability to be more photostable than organic dyes. Dihydrolipoic acid (DHLA)-capped cadmium selenide-zinc sulphide (CdSe-ZnS) QDs showed no loss in intensity after 14 h, and were nearly 100 times as stable as, and also 20 times as bright as, rhodamine 6G [9]. As discussed formerly QD's can be of certain use in areas where long-term monitoring of labelled substances is entailed. In time gated imaging QD's can be a matter of advantage as they are capable of long fluorescent lifetime even after excitation. The fast fluorescence emission of organic dyes upon excitation coincides closely with short-lived auto fluorescence background from many naturally occurring species, reducing the signal-to-noise ratio. On the other hand, QDs emit light with a decay time in the order of a few tens of nanoseconds (30–100 ns) at room temperature, which is slower than the auto fluorescence background decay, but fast enough to maintain a high photon turnover rate.

B. Quantum Confinement Effect And Size Tunable Properties:

Semiconductor materials that lie on the nanometre scale are nothing but the quantum dots. They obey the quantum mechanical principle of quantum confinement. QD does also have the ability to exhibit energy band gap which helps in determining the required wavelength of radiation absorption and emission spectra. The essential absorption and resultant emission wavelength depends upon the dot size. One of the tantalizing attribute of QD's is that by modifying the size and the chemical composition fluorescence emission can be tuned from the near ultraviolet, throughout the visible and into the near infrared (NIR) spectrum, spanning a broad wavelength range of 400–2000 nm [12-14].

Solid state physics most often divides materials into three conventional listing: conductors (metals), semiconductors and insulators deployed on electron conductivity. This conductivity of solid material is ascertained by the difference of energy level between the valence band and the conduction band. The highest electronic energy level that electrons occupy at room temperature is the valence band (figure 2). Likewise the lowest electronic energy level which is not engaged by any electrons is the conduction band. Either by acquiring energy thermally or by absorption of photon electron from the valence band may gain energy and enter the conduction band thus relinquishing a positively charged hole in the valence band. Thus the term bandgap energy was coined denoting the difference in energy between valence band and conduction band and is expressed in electron volts [eV]. It specifies that the energy must be gained by the electron to enter the conduction band.

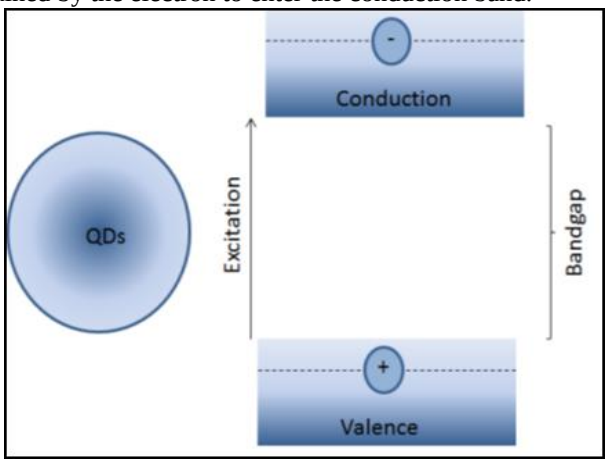

Fig. 2: A schematic representation of the valence and conduction band of QD nanoparticles.

The raised electron and the hole taken as a pair are called an "exciton". Once in the excited state (electron in conduction band), a conduction-band electron may relax back to its ground state in the valence band through radiative recombination with a hole, resulting in the emission of a photon with the same energy as the bandgap. Among the many possible fester processes, light emission is of both elemental and practical value. QDs as semiconductor materials exhibit size-dependent energy state due to the confinement of the charge carriers (electrons-holes) in three dimensions. As illustrated in figure 3 the size of the quantum dot determines the band gap [17]. As the size of the QD drops down the band gap rises and ultimately emerging in the shorter wavelength of light. However bandgap is not only decided on the size but also on the particle composition [16] which also is a parameter to remould the bandgap of a semiconductor. Thus substantial attempts are made to engineer fluorescence emitters to tune bandgaps as it helps in deciding the fluorescence emission wavelength of semiconductor material.

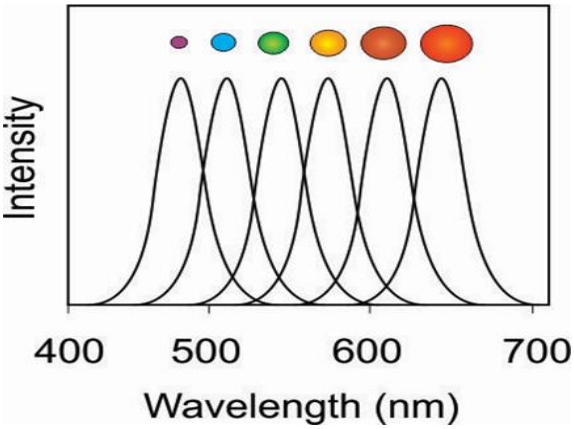

Fig. 3: Emission spectra as a function of size for the same quantum dot material. The colored spheres represent the size decrease of the particles

III. SYNTHESIS

Diverse ways are used to manufacture QD's [18] but predominantly QD's use the procedure of top down and bottom up fabrication. Top down outlook includes methods such as Molecular Beam Epitaxy (MBE), Ion implantation, E-beam lithography and X-ray lithography. Top down processing for manufacturing Qdots starts with bulk semiconductor which is thinned initially. Very often E-beam lithography, reactive-ion etching, and/or chemical etching methodologies are practiced to achieve QD's of approximately 30 nm in diameter. Arrays of zero dimension dots are fabricated alternatively by focused ion or laser beams. However a considerable disadvantage of these processes is of embodiment of impurities into the Qdots and structural imperfections by patterning [18]. The second approach for fabrication is bottom-up which uses several varied self assembly techniques to synthesize QD's. Colloidal QD's are generally fabricated by self assembly in the solution following a chemical reduction [19-22]. The self assembly techniques can be predominantly subdivided into (a) wet chemical and (b) vapour phase methods [18].

1) Wet chemical method: This process follows the mainstream precipitation method with attentive control of parameters for either single solution or mixture of solutions. The precipitation process at all times involves both nucleation and limited growth of particles. Nucleation can be grouped as homogeneous nucleation that occurs when solute atoms or molecules combine and reach a critical size without the assistance of pre-existing solid interface, heterogeneous nucleation or secondary nucleation [23]. Micro-emulsion, sol-gel [24-26], competitive reaction chemistry, hot-solution decomposition [27-29], sonic waves or microwaves [30] and electrochemistry are other wet chemical methods.
2) Vapour-phase method: For constructing QD's with vapour-phase method, layers are grown in an atom-by-atom process because of this self assembly of QD's occur on substrate without any patterning [31-33]. Self-assembly of nanostructures in material grown by MBE, sputtering, liquid metal ion sources, or aggregation of gaseous monomers are generally categorized under vapour-phase methods. MBE has been mainly used to self assemble QDs from III-V semiconductors and II-VI semiconductors using the large lattice mismatch, e.g., InAs on GaAs has a 7% mismatch and leads to SK growth.

IV. TOXICITY

Toxicity of nanomaterials is exceptionally complex due to the diversity of materials. To use QD's in biomedicine and clinical sector it is of utmost importance to deal with the biocompatibility and toxicity [34-36]. The toxicity of QD's is checked on factors such as QD's size, charge, concentration, outer coating bioactivity (capping material, functional group) as well as oxidative, photolytic and mechanical stability. Cadmium is the most commonly used QDs at present. Although this element is incorporated into the core of the nanocrystal, surrounded by inert zinc sulphide, and encapsulated within a stable polymer, it is still unclear if this toxic ions can be used as clinical contrast agents. Therefore, despite potential biomedical applications of QDs concerns persists about their safety.

In vitro studies have shown that QD toxicity arises from several factors such as chemical composition, size, shape, surface charge, surface coating, and dose as mentioned earlier. Their ability for photo-induced formation of reactive oxygen species (ROS) and nanoparticle aggregation are other parameters involved in QDs toxicity [37-42]. Toxicology data derived from in vitro studies may not reflect the response of a physiological system to an agent. Animal model is the preferred system for the toxicological assessment of a novel agent. Therefore comprehensive in vivo toxicity evaluation of QDs is crucial for their clinical translation. In vivo toxicity is determined by several factors including dose, route of administration, metabolism, excretion rate, and immune response. Chemical composition, size, shape, aggregation and surface coating are also involved in QD-induced toxicity.

V. BIOMEDICAL APPLICATIONS

A. Drug Delivery and Cancer Therapy:

QDs provide multifaceted platform for engineering traceable drug delivery systems with potential for improving treatment of cancers. The mechanism of delivery of QD and drug formulations to tumor cells depends on the architecture and properties of the nanostructures. Several rules must be considered in preparing QD and drug nanoparticle formulations for targeted therapy in vivo: (i) the nanoparticle surface must be functionalized with targeting ligands for specific delivery to tumor cells and must allow the drug to be delivered together with the carrier. (ii) Minimum size of the nanoparticle to allow excretion from the body. (iii) The drug molecules must be confined within the nanoparticle delivery system to prevent any harmful effects to the normal tissue. (iv)The surface of QDs must be passivated with a long lasting biocompatible polymer to prevent degradation or breakdown of the QDs upon encounter with the internal biological environment. Two ways can be used to integrate QDs and drug molecules into a nanoparticle formulation: (i) conjugating or linking drug molecules to the QD surface, followed by delivery of drug-conjugated QDs to specific sites and subsequent release of the drug molecules from the QD surface in response to local biological conditions such as pH or the presence of enzymes or (ii) loading the drug in a polymer nanoparticle system that also contains either hydrophobic or hydrophilic QDs, depending on the type of polymer particle used to encapsulate them. The entire QD/drug nanoparticle system is delivered to the desired organ or tissue, and the drug molecules are either released when the polymer particle is degraded at low pH or simply diffuse out from polymer particle. For example, figure 4 shows the first approach, demonstrating the synthesis of QD−aptamer (Apt) −doxorubicin (Dox) conjugates (QD−Apt (Dox)) as a complex conjugate for targeted cancer imaging, therapy, and sensing [1].

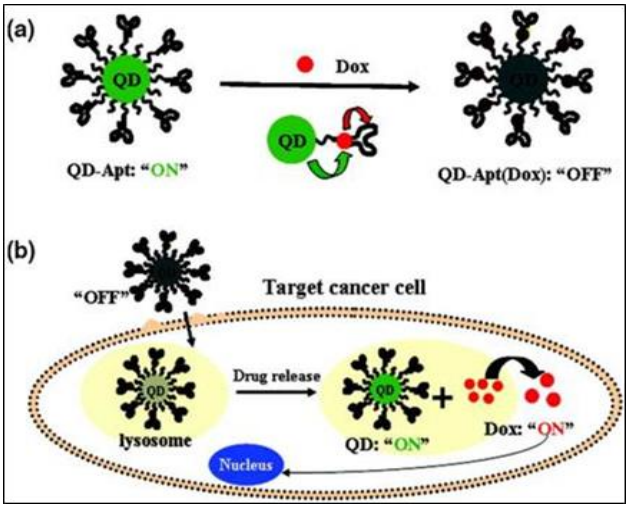

Fig. 4: Preparation of Quantum Dot/Drug Nanoparticle Formulations for Traceable Targeted Delivery and Therapy

B. Dna Labelling:

It is very prudent to observe and conceptualize the synergy of multiple protein or DNA sequences present in cells or tissues to perceive the complexity of synergy in biological molecules. A policy for the same is quantitatively imaging of biochemical contents in organisms. For this diverse receptors (ligands) on cells can be simultaneously screened and analyzed by using a confocal micro-spectrophotometer. This could provide a direct approach to identify sets of DNA sequencing that correlate with presence of certain diseases [43]. Existing ways of labelling and visualizing DNA and protein molecules rely on the light-emitting properties of a limited group of radioactive elements, chemical dyes and protein molecules. These labelling techniques have several drawbacks such as radioactive markers have short life spans; organic dyes have a limited number of colours and quickly lose their glow. While there is great demands for more reliable, robust labelling fluorophores in biomedical research to study real-time imaging and quantitative determination of multiple-molecule types present in cells or tissues. The luminescent QDs can overcome the functional limitations encountered with normal chemicals and organic dyes. Since QDs are highly stable against photo bleaching and have narrow, symmetric emission spectra. The emission wavelength of quantum dots can be continuously tuned by changing the particle size or composition. Thus a single light source can be used for simultaneous excitation of all different-colored QDs. These novel optical properties of quantum dots make them ideal fluorophores, sensitive for multicolour and multiplexing. Thus QDs expand the possibilities for use in fluorescence imaging of biological analysis and medical detection. The fluorescence intensity of the CdS-DNA decreases with the increasing temperature and are successfully used in medical diagnosis and quantitative mode of fluorescence in-situ research and biologic analysis [44].

C. Photodynamic Therapy:

Photodynamic therapy (PDT) is used to treat a wide range of medical conditions, including age-related muscular degeneration and cancers. It involves three key components, a photosensitiser, light source and tissue oxygen (atomic oxygen). The combination of these components leads to the chemical destruction of any tissues. QDs are used to generate the atomic oxygen, which is largely taken in by the cancer cells than a healthy tissue. Hence, only the cancer cells are destroyed when exposed to a laser radiation in the presence of QDs. Further it is noted that the remaining dye molecules sensitive to the daylight exposure. This can last up to many weeks, which can be avoided by the hydrophobic version of the dye molecule [45]. The potential for photosensitization in PDT can be compared with conventional photosensitizing organic dyes. Conventional photosensitizer is based on organic dyes that are efficient generators of cytotoxic reactive oxygen species. While unique optical properties of QDs and its conjugates could provide a new class of materials for versatile therapeutic applications. QDs decorated with iridium complexes that have potential in photodynamic therapy (PDT) treatment of cancer due to high enough emission from the luminescent CdSe/ZnS quantum dot. Further the iridium complexes are also enables to sensitize oxygen molecules to produce singlet oxygen on exposure to light. The quantum dots of CdSe have been also reported for use in these lines but fairly low levels of singlet oxygen generated [46]. The applicability of semiconductor QDs in photodynamic therapy (PDT) was evaluated by studying the interaction between CdSe QDs with a known silicon phthalocyanine PDT photosensitizer. This revealed that the QDs could be used to sensitize the PDT agent through a fluorescence resonance energy transfer (FRET) mechanism or interact directly with molecular oxygen via a triplet energy-transfer process (TET). Both mechanisms result in the generation of reactive singlet oxygen species that can be used for PDT cancer therapy [47].

D. Diagnosis and Imaging:

The distinctive properties of QD's makes them fit for in vivo and in vitro in detection of varied biomolecules, micro-organisms and toxins. Several different kinds of organic dyes are used in present-day biological analysis. As more flexibility is required as traditional dyes are incompetent to meet the expectations. Thus QD's came into picture to fill up the role as upmarket to traditional organic dyes on several counts, like brightness (owing to the high extinction co-efficient combined with a comparable quantum yield to fluorescent dyes), stability (allowing much less photo-bleaching). As equated to traditional fluorescent dyes, reports reveal that QD's are 20 times brighter and 100 times more stable thereby allowing data acquisition of several successive focal-plane images that can be restored into high-resolution 3-D image. Another potential application of quantum dots as a result of its photostability is real-time tracking of molecules and cells over long duration. Scientists have observed that existence of QD's in lymph nodes of mice for more than four months theorized the utility period of QD's. One more probable application of QD's is fluorophore for intra-operative detection of tumor with the help of fluorescence. The discrete nanocrystals that are encased in phospholipids block- copolymer as micelles are proved to be stable, non-toxic and applicable for vitro and in vivo imaging. The QDs conjugated to biomolecules (DNA) is nanocrystal micelles and acted as in vitro fluorescent probes to study specific complementary sequences. In real time imaging propensity to picture single-cell migration is a prime necessity in several research areas namely embryogenesis, cancer metastasis,

stem-cell therapeutics and lymphocyte immunology. In this factor QD's technology are far superior to current methods like siRNA. In addition, QD's are being used for tumor targeting under in vivo conditions where two schemes for targeting the cells are implemented a.) Active targeting and b.) Passive targeting. In the case of active targeting, QDs are functionalized with tumor-specific binding sites to selectively bind to tumor cells but the passive targeting utilizes the enhanced permeation and retention of tumor cells for the delivery of QDs probes. The fast-growing tumor cells typically have more permeable membranes than healthy cells, permitting the leakage of small nanoparticles into the cell body. Another principle part of cell's metabolism is protein therefore to analyse its structure and functionalities is of vital importance [48]. To examine the protein-protein interaction gold nanoparticles have been commonly used in immune histochemistry but back to back detection capability is not possible. In other prospective the tissue-based analysis of protein expression provides crucial diagnostic and prognostic information. Through antigen-antibody interactions using antibodies labelled with fluorescent dyes, enzymes, radioactive compounds, or colloidal gold the detection of analytes in tissue can be accomplished. Multiplex tissue analysis, in which two or more analytes are detected simultaneously, is a powerful approach to study co-expression and spatial distribution of protein expression, while it consumes fewer tissue samples. Composite organic-inorganic QDs are novel optical labels for detection of biomolecules for detection of proteins in human tissues. Sun et.al has detected two analytes in a direct binding assay on silver nanoparticle encapsulated composite [49].Further, visual analysis of biomolecules is an integral part of biological research and has been carried out by tagging of nucleotides and proteins with traditional fluorophores which are limited in their application due to their properties such as photo bleaching, spectral overlaps, and operational difficulties. However, QDs have emerged as a superior alternative and poised to change the world of bio-imaging [50]. QDs have offered a benefitting reply to the constraints for fluorophores traditionally used for tagging bio molecules. They are made up of tiny semiconductor crystals comprise of promising feature like brightness and photostable more sensitive and multiplex coding. They have a broad excitation spectrum but a narrow emission at wavelengths controllable by the size and composition of a core. The non-targeted infrared emitting QDs cores are suitable for tumor optical imaging in mice after intravenous injection of QDs. The preliminary experiment, were shown for generating a reasonable signal to noise image. The bio-distribution pattern is determined from the optical image shows favourable clearance of the non-targeted through the lymphatics, kidneys and bladder. No uptake in the tumor was observed, suggesting the next round of imaging to be done with tumor targeted will have minimal background signal within the tumor. The development of QDs as non-invasive optical molecular imaging probes will have a great impact on the early detection, diagnosis and treatment monitoring of cancer [51]

VI. Conclusions

This upcoming class of nanomaterials is thus studied along with its optical properties and basic concepts of QD's. The various synthesis methods followed for the fabrication of QD's with it toxicity impact is explained in the review. Furthermore explaining the biomedical applications of these 0-D nanomaterials is reported.

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