Micro- and Nanotechnologies-Based Product Development

5 Pharmaceutical and Biomedical Applications of Multifunctional Quantum Dots

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5.1 INTRODUCTION

Conventional formulations developed by pharmaceutical industries are in abundance, though with limited efficacy, poor permeation, decreased bioavailability and toxicity (Uehara et al. 2010). Nanotechnology is a tool for delivering drugs at specific target sites using intelligent and smart nanocarriers having well-defined sizes and shapes (Lalu et al. 2017; Tekade et al. 2017). Nanostructured materials possess the ability to bridge the gap between molecular and bulk levels and therefore create new avenues for a wide range of applications in biology, electronics and optoelectronics (Maheshwari et al. 2015; Sharma et al. 2015; Maheshwari et al. 2018; Moondra et al. 2018). Based on particle size, these nanostructures can be categorised as zero-dimensional or quantum dots (QDs), one-dimensional or quantum wires, and two-dimensional or quantum wells (Bera et al. 2010).

QDs, also known as semiconductor nanocrystals, pertain to unique electronic and optical properties, including multiplexed capabilities, long-term photostability, high signal brightness, simultaneous excitation of multiple fluorescence colours and size-tunable light emission (Jin et al. 2011). QDs are nanometre-sized semiconductor structures with dimensions smaller than the de Broglie wavelength (Mandal and Chakrabarti 2017). Nanometre size increases the particle surface area-to-volume ratio, which further enables surface modifications to ameliorate reactivity, solubility and biocompatibility (Wagner et al. 2019). QDs are proven to be integrated with a range of applications in biomedical sciences, including fluorescent assay for drug discovery, bioimaging, detection of disease, intracellular reporting and protein tracking (Rosenthal et al. 2011). Moreover, these nanometre-sized QDs also overcome severe toxicity, decrease effective dose and increase sensitivity (Wagner et al. 2019).

5.2 HISTORY OF QDs

At the end of 1970, during the crisis of petroleum, researchers aimed at discovering alternatives for solar energy conversion. This provokes the investigators to synthesise semiconductor crystals in solution and screens their optoelectronic properties. Meanwhile, they observed blueshift with a decrease in nanocrystal size and explained quantum confinement effect. Typically, the studies on QDs were initiated in physics and then emerged through medical and technical fields. Of note, quantum oscillator, transistor, multispectral fluorescent dye imaging, filters, detectors, data analysis technique and QD light imaging device are some inventions in the field of physics (Bera et al. 2010; Efros and Nesbitt 2016). Interestingly, the outstanding and unique properties of QDs make them novel drug delivery and targeting approaches (Figures 5.1 and 5.2).

5.3 TYPES OF QDs

These are nanoscale man-fabricated crystals that can convert light spectrum into diverse colours. According to the size of these QDs, every dot emits a different colour. QDs can be classified into distinct types based on their composition and structure, such as core-type QDs, core-shell QDs and alloyed QDs (Liu and Su 2014). A typical structure of QDs is presented in Figure 5.3.

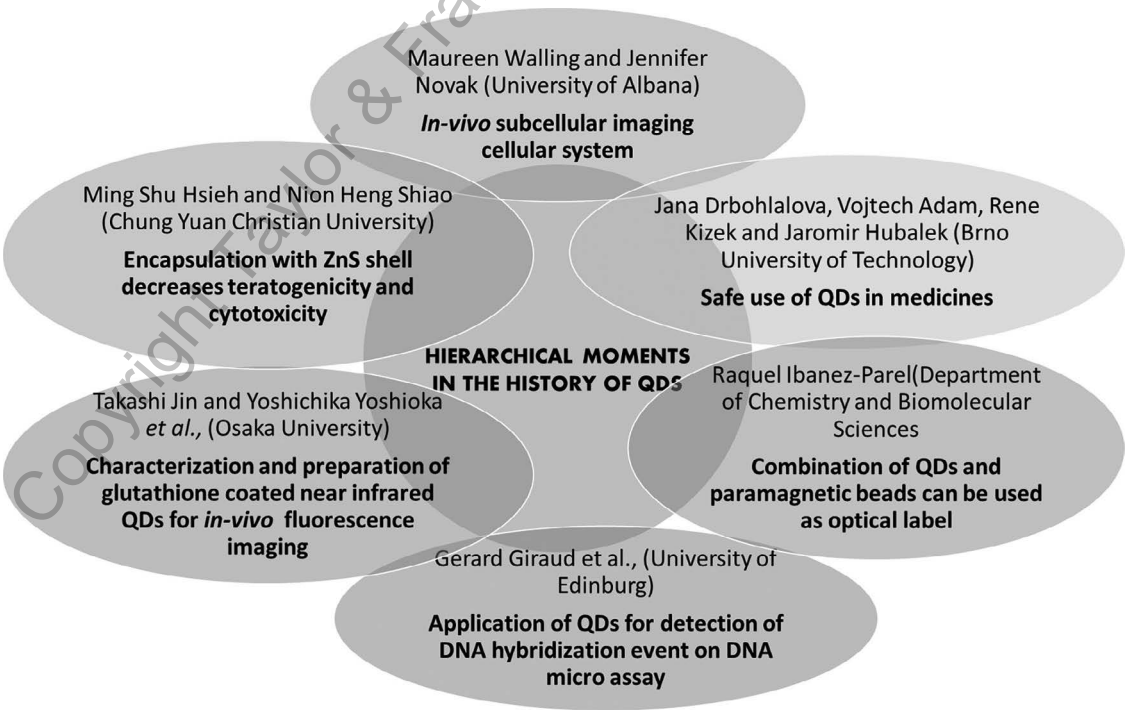

FIGURE 5.1 Hierarchy in the history of QDs. Hierarchical moments marked in the journey of development of QDs by global universities including its application in medicine as imaging system, diagnostic tools and optical labelling.

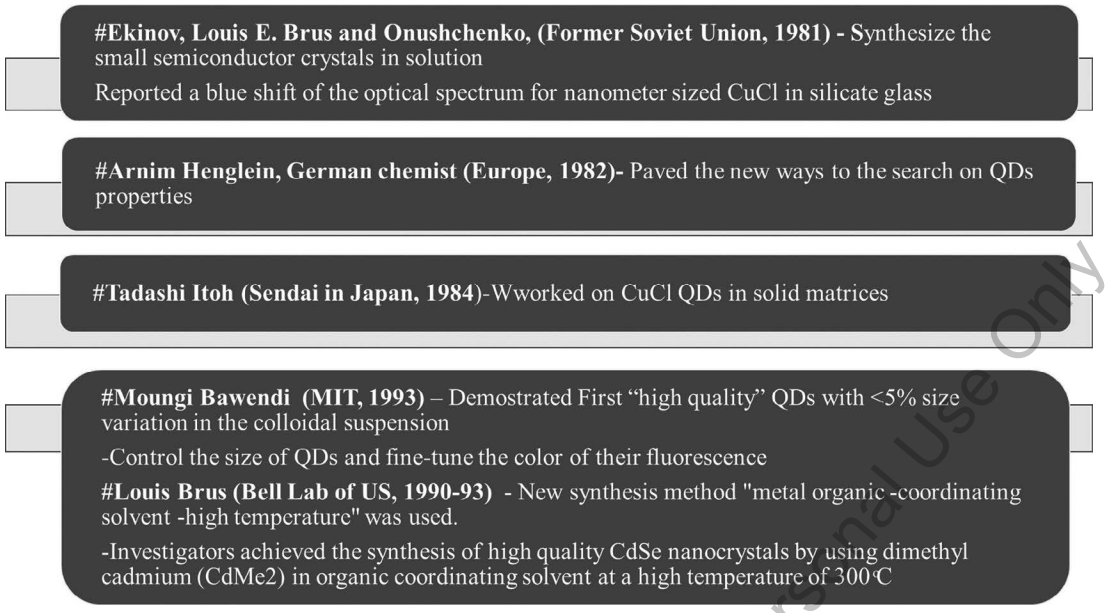

FIGURE 5.2 Historical representation of QDs. The figure highlights the contribution of eminent scientists globally for historical development of QDs possessing outstanding and unique properties that make them a novel drug delivery material in targeting and delivery.

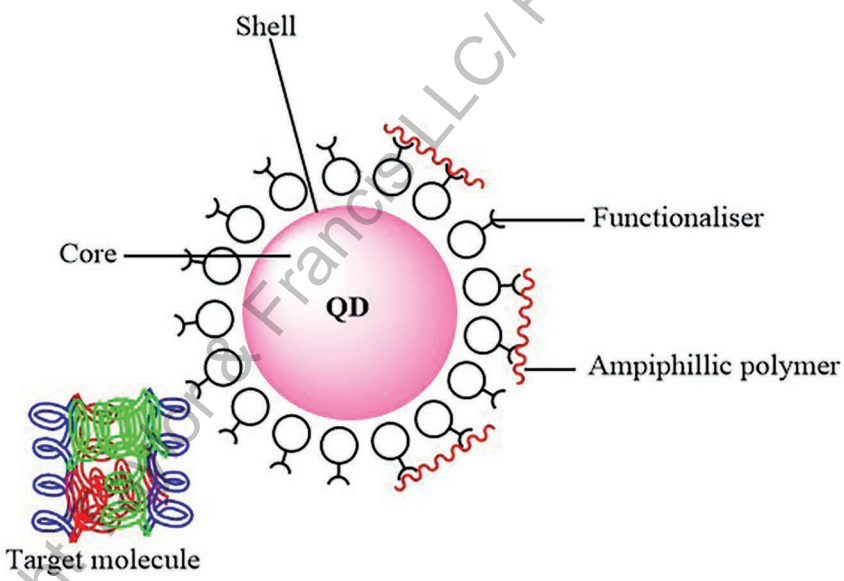

FIGURE 5.3 Structure of a typical QD. Diagrammatic representation of a basic QD with core (semiconductor such as CdSe or CdTe), shell (such as ZnS), amphiphilic polymers (such as PEG) and target molecules such as peptides or antibodies.

5.4 SYNTHESIS OF QDs

5.4.1 Organic-Phase Method/ Organometallic Chemistry Method

The organometallic chemistry method is considered as the most crucial method for the synthesis of regular and uniform core-structured monodisperse QDs with high quantum yield in non-polar organic solvents (Aswathi et al. 2018). Different sizes of QDs can be obtained by varying temperature and reaction time conditions. In general, bis(trimethylsilyl)selenium ($(TMS)_2Se$) and Me_2Cd are two profusely used organometallic precursors. Monodisperse CdSe can be obtained based on the pyrolysis of organometallic reagents by injection into a hot coordinating solvent between 250°C and 300°C. The adsorption of ligand such as tri-n-octylphosphine oxide (TOPO) leads to annealing of cores in coordinating solvents (Jin et al. 2011).

5.4.2 Water-Phase Method/Aqueous Solution Method

The aqueous solution method is a cost-effective and eco-friendly procedure of QD synthesis. The technique has direct applications in biological research without the involvement of the ligand exchange procedure. In general, glutathione (GSH), 3-mercaptopropionic acid (3-MPA) or other hydrosulfyl-containing materials are the commonly used ligands for the production of CdTe QDs in aqueous solution. In addition to this, ionic perchlorates such as Al_2Te_3 and $Cd(ClO)_4 \cdot 6H_2O$ are used as the precursors. Thiol-capped CdTe QDs were the first synthesised aqueous dispersed QDs; however, they showed low quantum yields, poor stability and broad size distribution when compared with the QDs produced by the organometallic method (Jin et al. 2011; Aswathi et al. 2018).

5.4.3 Hydrothermal and Microwave-Assisted Irradiation Methods

Hydrothermal and microwave-assisted irradiation methods are used to produce QDs with high quantum yields and narrow size distribution. Moreover, these methods also reduce the reaction time and surface defects generated during the growth process of QDs due to high pressure. In brief, all reaction reagents are heated at high temperatures up to supercritical temperature into the hermetic container. In the microwave-assisted irradiation method, microwave irradiation is considered as a heating source which aids in optimising synthesis conditions. An increase in heat from this system produces homogenous QDs with high yield (17%) (Jin et al. 2011; Aswathi et al. 2018). Different synthesis methods of QDs are presented in Figure 5.4.

5.4.4 Laser Ablation Techniques for QDs

This method is reported as a clean technique for the fabrication of QDs due to less waste production. In this method, nanoparticles are fabricated by employing high-energetic laser light on metals or crystals. Anikin et al. used laser of Cu to obtain QDs of CdS/ZnSe in open air by ablating CdS/ZnSe under a slim liquid layer just above the semiconductor surface. They employed different liquid mediums such as isobutanol, diethylene glycol, ethanol and dimethyl sulfoxide (Horoz et al. 2012).

5.4.5 Molecular Beam Epitaxy (MBE) and Nanopatterning for QDs

The MBE is an epitaxial growth technology that relies on the interaction of molecules of thermal energy and beams of atoms on a heated crystalline surface along with ultra-high-vacuum conditions. One of the latest methods for QD fabrication is droplet epitaxy (DE). This method is fully

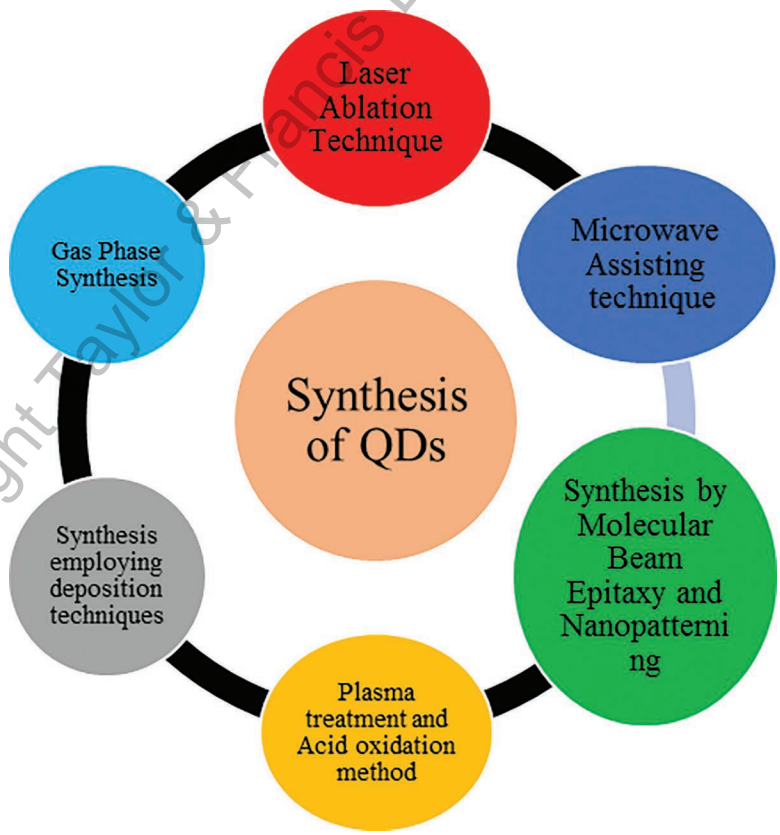

FIGURE 5.4 Various approaches for the synthesis of QDs. Diagrammatic representation of multiple synthesis approaches considered during the development of QDs.

compatible with MBE technology and produces various zero-dimensional quantum structures such as double ring-like QDs and inverse QDs. Claro et al. (2019) reported the development of self-assembled Bi_2Se_3 QDs by MBE on GaAs substrates employing the droplet epitaxy technique (DET) (Claro et al. 2019).

5.5 PROPERTIES OF QDs

Structurally, QDs are composed of semiconductor core (groups III–V and II–VI), enclosed within a shell formed of another semiconductor material (Figure 5.3). Notably, QDs have five unique properties that give them their distinct capabilities. Firstly, the diameter of QDs ranges from 2 to 10 nm comprising approximately 200–10,000 atoms. Secondly, QDs discern themselves with other fluorescent proteins and organic dyes in offering unique electronic and optical properties for targeted drug delivery and imaging (Bajwa et al. 2016).

QDs are exceptionally bright due to high fluorescent quantum yields and possess large absorption extinction coefficients. Thirdly, electronic and optical properties include composition- and size-tunable light, narrow and Gaussian emission spectra, signal brightness, multiple fluorescent colour excitation, and resistance to photobleaching, which enables extended dynamic imaging. Fourth, QDs blinking, it is interesting that different coloured QDs can be excited simultaneously with minimal spectral overlapping, with a single light source, which provides significant advantages for multiplexed detection of target molecules (Peng and Li 2010; Rosenthal et al. 2011). Despite the hydrophobic nature of QDs, the large surface area required for attaching biofunctional molecules necessitates solubilisation of QDs. Fifth, they are photochemically robust and inorganic (Rosenthal et al. 2011). The well-understood and well-documented property is an inverse relationship that exists between energy band gap and nanocrystal size. In simpler words, with the increase in the energy band gap, the nanocrystal size decreases and the corresponding emission/excitation wavelength also decreases. This concept is known as the quantum size effect (Wagner et al. 2019).

Advantages and properties of QDs in biological and chemical research concerning their spectral overlapping, low signal intensity and photobleaching distinguish them significantly from green fluorescent proteins and traditional fluorescent organic dyes (Jin et al. 2011). Also, QDs resist photobleaching for longer fluorescent durations and show minimal cytotoxicity as compared to an organic fluorescent dye. Due to reduced photobleaching tendencies, QDs can preserve and image the number of samples a number of times (Amaral et al. 2020). Such properties of QDs considered being potential candidates as labels and luminescent probes in biological applications ranging from biological imaging, diagnosis of disease to molecular histopathology. Numerous studies have also been reported on the use of QDs for *in vitro* and *in vivo* imaging (Jin et al. 2011).

5.6 BIOMEDICAL APPLICATIONS OF QDs

For the most promising application of QDs in biological imaging, several advanced studies have focused on developing fluorescent probes. QDs possess the ability to play a significant role in cell biology, *in vivo* and *in vitro*, and have even replaced a lot of fluorescent dye molecules when coupled with biological molecules, as shown in Table 5.1 (Jin et al. 2011; Zhao and Zeng 2015).

TABLE 5.1
Applications of QDs in Molecular, Cell, Tissue and Animal Model Cancer Imaging

Category	Components	Findings	Future Needs	References
In vivo cell imaging	Fibrous phosphorus quantum dots (FPQDs)	FPQDs were used for live imaging using simple fluorescent microscopy with 4′,6-diamidino-2-phenylindole (DAPI) filter Bioimages of human adenocarcinoma cells were acquired	–	Amaral et al. (2020)
	Streptavidin-conjugated green QDs (QD525), Tetrazine-conjugated red QD (QD625) and transcription activator-like effector (TALE)-labelled QDs	Single-genome loci were imaged *in vivo* by combining QDs with TALEs labelling technique	Colocalisation microscopy can then be employed to examine the bound QD-TALEs The ultrasensitive analysis may contribute to the study of chronic HIV-1 infections, as well as virus detection and treatments at the live-cell level	Ma et al. (2017)
	Biocompatible heavy metal-free/cadmium-free QD nanoparticles (bio CFQD® nanoparticles)	Effectiveness and applicability of novel biocompatible water-soluble indium-based QDs for *in vivo* axillary lymphatic mapping and lymph nodes in a prostate cancer mouse model	Due to the absence of toxic elements in bio CFQD® nanoparticles, they may further be used in biomedical applications, but after additional work to study long-term toxicity	Yaghini et al. (2016)

(*Continued*)

TABLE 5.1 (*Continued*)
Applications of QDs in Molecular, Cell, Tissue and Animal Model Cancer Imaging

Category	Components	Findings	Future Needs	References
In vitro imaging	PEG-functionalised QDs	Non-aggregated QDs were internalised by microglia *in vivo* and in slices, irrespective of the brain-relevant platform and surface chemistry. Thus, providing a brain microenvironment allows the development of QD-based imaging probes that enables targeting an interested region in the CNS	Results guided to further engineer candidate QD-based imaging probes for neurological applications	Zhang et al. (2019)
	QD-conjugated probes	Cell clone formation assay based on QD molecular imaging provided a novel method to understand the proliferative feature and morphological characteristics of cancer cells providing an insight onto tumour biology	There is theoretical feasibility and technical possibility to develop a differentiation strategy which may allow for the control and coordination of cancer cells	Geng et al. (2016)
Drug delivery	GQD-PEG-BFG-Pyr-RF and GQD-Pyr-RF	GQD-PEG-BFG-Pyr-RF possesses less cytotoxic effect, and subsequently, its effect on three cancer cell lines was compared with the effect produced by anti-cancer drug (GQD-Pyr-RF) which was similar to nanovector lacking targeted riboflavin ligand (GQD-PEG-BFG)	The therapy opens new possibilities for poorly water-soluble anti-cancer drugs	Iannazzo et al. (2019)
	FA-PEG-cGQDs-MTN nanosystem	The nanodelivery system showed low systemic toxicity, improved anti-tumour ability and targeting capacity in a live animal without the presence of systemic adverse effects	Despite the development of such a nanosystem, clinical settings should be carefully considered by using clinical tumour models Also, mimetic membranes should be used to improve the immune evasion capabilities	Li et al. (2019)
	Carboxylated PEGylated QDs and QD-encoded microcapsules	The conjugate of a monoclonal antibody targeting HER2 and microcapsule provided specific and sensitive antibody-mediated binding of microcapsules with living cancer cells	QD-encoded microcapsules can be biocompatible fluorescent agents for live-cell targeting, and serve as the basic platform for further development of targeted systems for diagnosis and therapy of a variety of tumour entities	Nifontova et al. (2019)
Cell labelling	Qdot® 625 ITK™ carboxyl QD	The carboxylated QDs can used as an effective and non-specific dye for labelling bone marrow MSCs	Results showed promising future in tissue engineering, anti-cancer drug delivery, cellular therapy and fundamental stem cell biology	Kundrotas et al. (2019)
Biosensors	Fe_3O_4/CdSe composite QD	For designing magnetic Fe_3O_4/CdSe QD-based electrochemiluminescence for assay of cancer cells using cyclic amplification technology	The design and development of magnetic QD-based ECL have the potential in clinical applications	Jie et al. (2013)

5.6.1 *In Vivo* Cell Imaging

In recent years, applications of QDs have been noticeable in the field of cellular biology and living cells (Table 5.1). Weng et al. aimed at incorporating exclusively luminescent QDs in immunoliposomes for imaging, diagnosis and treatment of cancer. They synthesised QD-conjugated immunoliposome-based nanoparticles by inserting anti-HER2 scFv in HER2-overexpressing MCF-7/HER2 and SK-BR-3 cells. They showed that QD-conjugated immunoliposome-based nanoparticles increase the circulation of QDs in athymic mice. Also, plasma $t_{1/2}$ was found to be ~2.9 h when compared with free QDs with $t_{1/2} < 10$ min. *In vivo* fluorescent imaging was used for confirming localisation of QD-conjugated immunoliposome-based nanoparticles in MCF-7/HER2 xenograft models (Weng et al. 2008).

QDs have a unique property that with increasing separation between emission and excitation wavelength, the absorbance of QDs also increases. Based on this, much of enthusiasm is generated for applying QDs in *in vivo* stems, since photon yield should be proportional to the integral of the broadband absorption. Lim et al. hypothesised fluorescent QDs to be excellent contrast agents for biomedical imaging and assays. Based on a validated mathematical model, they explored the effects of tissue thickness, water-to-haemoglobin ratio, the wavelength dependence of

scattering, tissue scatter and tissue absorbance on the performance of QDs. In conclusion, the excitation wavelength remains constrained and should be selected carefully based on the particular application of QD, when embedded *in vivo*. Near-infrared QD was produced and optimised for imaging surface vasculature with a silicon CCD camera and white light excitation. They were also used for imaging coronary vasculature *in vivo* and may have applications in designing fluorescent QD contrast agents optimised for specific biomedical applications (Lim et al. 2003).

Even after specific selection of emission and excitation wavelengths, many factors such as pharmacokinetics, toxicity, photostability, chemical stability and quantum yield remain unanswerable for their impact on QDs for biomedical application. Since, no reports were published for screening effect of QDs on such factors, after its *in vivo* administration into tumours to map sentinel nodes. Ballou et al. used oligomeric phosphines for capping QDs, which preserved photostability for at least initial contact hours of QDs with plasma. This paramount imparted aqueous stability, solubility, maximised quantum yield and minimised non-specific tissue interactions. They also investigated that retention and binding in the lymph node are affected by changing the surface charge of PEG-conjugated QDs (Ballou et al. 2007).

Amaral et al. developed a facile solution-based method for synthesis of fibrous phosphorus QDs with a height of 2.7 ± 1.3 nm and an average size of 3.8 ± 0.9 nm. Remarkable stability was obtained along with fluorescence properties in the indigo-blue region. Further, these fibrous phosphorus QDs were used as fluorescent labels in live bioimaging of human adenocarcinoma cells (Amaral et al. 2020).

Small peptides known as cell-penetrating peptides (CPPs) are able to traverse cell membrane and deliver a variety of molecules within a living cell. Such agents tend to deliver QDs across the membrane with minimal toxic and non-specific absorption effect. Liu et al. designed polyethylene glycol lipid-coated carboxyl-functionalised, monodisperse and water-soluble indium phosphide/zinc sulphide (InS/ZnS) QDs. They characterised cellular internalisation and physicochemical properties of CPP/QInP complexes and carboxyl- and PEG-bifunctionalised indium phosphide (InP)/ZnS QDs (QInP). These CPP/QInP complexes were efficient in delivering QInP in human A549 cells. This was evident when QInP (<1 M) and CPP/QInP complexes (500 nM) did not affect cell viability. Conclusively, they reported that PEGylated and carboxylated biofunctionalised QInP were biocompatible nanoparticles with potential applications in drug delivery and bioimaging studies (Liu et al. 2013).

Ma et al. designed a strategy for live-cell imaging of single genomic loci associated with cellular functions, pathogenic infections or genetic diseases. They combined the QD labelling technique with transcription activator-like effectors (TALEs) which specifically targeted HIV-1 pro-viral DNA sequences. Besides, two bio-orthogonal ligation reactions were used for labelling with varied colours. The first TALE was well labelled with tetrazine-conjugated red QD via the Diels–Alder cycloaddition and fused to short LplA acceptor peptide. The second TALE was labelled with streptavidin-conjugated green QDs (QD525) and merged with AP tag. Thus, TALEs labelled with QDs (QD-TALE) entered the cell nucleus via nuclear localisation sequence of TALE, identified single HIV-1 pro-viral loci by providing fluorescence and mapped in live U1 cells (Ma et al. 2017).

Yaghini et al. evaluated novel biocompatible heavy metal-free/cadmium-free QD nanoparticles (bio CFQD® nanoparticles) with good photoluminescence quantum yield. They evaluated the potential for mapping the lymph node by *ex vivo* imaging of regional lymph nodes after subcutaneous administration in rat paw. QDs were shown to accumulate selectively and quickly in thoracic and axillary regional lymph nodes according to chemical extraction and photoluminescent imaging methods (Yaghini et al. 2016).

Moreover, Zhao et al. proposed utilisation of QDs as spectra; converters that convert Cerenkov luminescence-blue emission to near-infrared light that is less absorbed or scattered *in vivo*. Cerenkov luminescence is an imaging modality that uses light produced during radioactive decay of clinically used isotopes. They showed that experimentation related to tissue phantom increases transmission intensity and penetration depth for Cerenkov luminescence in the presence of near-infrared QDs. They also developed three types of ^{89}Zr dual-labelled nanoparticles and near-infrared QDs based on polymeric nanoplatforms, nanoemulsions and lipid micelles, which enable co-delivery of radionuclide and QDs for maximised spectral conversion efficiency. Finally, they showed applications of self-illuminating nanoparticles for imaging tumours and lymph nodes in a prostate cancer mouse model (Zhao et al. 2017).

Apart from several advantages such as tunable emission and excitation spectra, low toxicity, surface functionality, photostability and photoluminescence required for *in vivo* imaging, no systemic evaluation of QDs in the brain microenvironment has been done yet. Zhang et al. investigated core-shell CdSe/CdS QDs' cellular uptake, colloidal uptake and *in vivo*, *ex vivo* and *in vitro* toxicity in the brain. They targeted red-emitting aqueous dispersible QDs with three surface functionalities: PEG-5000k-hydroxyl (PEG-OH), PEG-5000k-methoxy (PEG-OMe) and 3-MPA. They found that surface functionality plays an important role and is a dependable factor for QDs. PEG-conjugated QDs were protected from aggregation in neurophysiologically relevant tissues and fluids, allowing greater penetration. The behaviour of QDs differed in cultured slices as compared to monolayer cultures. Upon systemic administration, non-aggregated QDs were internalised by microglia *in vivo* and in slices, irrespective of brain-relevant platform and surface chemistry. Thus, providing brain microenvironment allows the development of QD-based imaging probes that enables targeting an interested region in the CNS (Zhang et al. 2019).

5.6.1.1 Synaptic Neurotransmission

Synapses are the medium for neurons to communicate, which shows enrichment for specialised receptors. Ehlers et al. reported that the mobility of GluR1 (glutaminergic receptor) was restricted to subregions of post-synaptic membrane. Such GluR1 mobility defines a new input-specific mechanism for AMPA receptor regulation in abundance (Ehlers et al. 2007).

Murphy-Royal et al. found that glutamate transporter (GLT-1) is enormously available on rat astrocyte. Surface modification of GLT-1 was sensitive to glial and neuronal activities and was decreased in the vicinity of glutamatergic synapses, leading to retention of transporter receptors. They gave first evidence for the physiological role of GLT-1 in shaping synaptic transmission, as improper diffusion of GLT-1 membrane via cross-linking in *in vivo* and *in vitro.* It increases time course for synaptic glutamate transmission (Murphy-Royal et al. 2015).

Extracellular space separates brain cells from each other and is vital as it provides a medium for drug and drug delivery vectors. Glia and neurons to access nutrients and chemical signalling necessitate diffusion within extracellular space. Thorne and Nicholson showed that water-soluble QDs and dextran with Stokes–Einstein diameter diffuse within the extracellular space of rat neocortex using integrative optical imaging. They were able to measure the width of extracellular space, i.e. 38–42 nm. The results improved modelling of neurotransmitter spread after ectopic release and spillover and established size limits for the diffusion of drug delivery vectors such as nanoparticles, liposomes and viruses in brain extracellular space (Thorne and Nicholson 2006).

Interestingly, knowledge regarding the application of QDs in presynaptic terminal has always been limited compared to diffusive behaviours of post-synaptic receptors. In general, disruption of actin induces a reduction in the diffusive behaviour of synaptic vesicle at synapse, while disruption of microtubules only decreases extrasynaptic mobility. Also, a significant increase in inter-boutonal and synaptic trafficking was produced by glycine-induced synaptic potentiation, which was actin- and NMDA receptor-dependent, while NMDA-induced synaptic depression reduced the mobility of synaptic vesicles at synapses. Results showed that SynaptopHluorin (sPH)-AP-QDs revealed unobserved trafficking properties of synaptic vesicles around synapses (Lee et al. 2012). Månsson et al. demonstrated novel application of streptavidin-coated CdSe QD-labelled isolated actin filament with preserved actomyosin functions. They evidenced that labelling covers both cross-linking of filaments and cargo (enzymes) transportation. Such photostable and bright QDs facilitate filament tracking and cargo detection for extended periods (Månsson et al. 2004).

Modi et al. demonstrated the use of high and small specific amine-modified QD-conjugated recombinant single-domain antibody fragment (VHH fragment) against green fluorescent protein to deliver information on diffusion of adhesive molecules at neurotransmitter receptor and growth cone at synapses. The results revealed that QD nanobodies were able to quantify the dynamics of neurotransmitter receptors at both inhibitory and excitatory synapses in *ex vivo* rat brain slices as well as primary neuronal cells. They demonstrated a strategy for multiple imaging of targeted/tagged proteins to monitor simultaneous behaviour, transport, diffusion and clustering (Modi et al. 2018). Caglar et al. developed a tool for analysing response of photoluminescence *in vivo* under AC and DC voltage changes and highlighted their imaging potential. They screened InP/ZnS and CdSe/CdS QDs to develop characteristics of PL/voltage on a chip. Such measurements with neuronal cells showed that QDs were used to track voltage changes sensitively. Also, CdSe/CdS QDs with more significant photoluminescence effect on depolarisation of membrane have lower cytotoxicity, which makes InP/ZnS more suitable for sensing in living (Caglar et al. 2019). In addition to this, Efros et al. examined the role of semiconductor trioctylphosphine oxide-coated QDs incorporated in liposome in addressing challenges of real-time optical voltage imaging (Efros et al. 2018).

5.6.1.2 Single Protein Tracking

QD single-particle tracking is a novel super-resolution imaging technique that utilises semiconductor nanocrystal QDs as a powerful tool for protein and lipid behaviour analysis in the membrane and as fluorescent probes (Bannai et al. 2020). However, targeting single-particle in rat hippocampus neuron with pH-sensitive QD probe reports movements of receptors on the surface. Taylor et al. evaluated a subpopulation of neuronal EphB2 receptors, which directed motion between the plasma membrane and synapses (Taylor et al. 2018). Varela et al. targeted dopamine receptors with functionalised QDs and performed single-molecule tracking *in vivo.* They also proposed a novel way to delocalised and non-inflammatory ways to deliver nanoparticles *in vivo* in the brain, which allowed to track and label genetically engineered surface of dopamine receptors in neurons, revealing regulation of activity and inherent behaviour in pathological and physiological animals (Varela et al. 2016).

5.6.1.3 Cell Tracking and Migration

Jayagopal et al. demonstrated a technique for *ex vivo* imaging of cellular recruitment in atherogenesis which utilises colour-code cell types to QDs within lesion area. It's well known that atherosclerosis progression is associated with infiltration of leucocytes within lesions. They coated QDs with fluorescently labelled immunomagnetically isolated macrophages/monocytes and T lymphocytes to maurocalcine (CPPs). QD–maurocalcine bioconjugates efficiently labelled both cell types with preserved cell viability and efficiency and did not disturb native leucocyte functionality in endothelial adhesion assay and cytokine release. They reinfused QDs–macrophages/monocytes and T lymphocytes in the ApoE-deficient mouse model of atherosclerosis and further observed that within two days of injection, the QD-labelled cells were visible in atherosclerotic plaque.

High signal-to-noise (S/N) ratio imaging of multiple biomarkers and cell types within the same specimen was enabled by this method of tracking leucocytes in lesions. Further, it also possesses great applicability in investigating the role of distinct circulating leucocyte subsets in the development and progression of plaques (Jayagopal et al. 2009)

Bilen et al. evaluated the feasibility of time-resolved fluorescence spectroscopy (TRFS) and scanning acoustic microscopy (SAM) in the characterisation of atherosclerotic plaque. They performed dual-modality imaging of human carotid atherosclerotic plaque, where they showed that acoustic impedance values were statistically lower in collagen-rich regions compared to calcified areas. They involved CdTe/CdS QDs for atherosclerotic plaque imaging using TRFS and showed a difference in fluorescence lifetime values of QDs in both the regions. Where TRFS provides information regarding the molecular environment of plaque, SAM highlighted the mechanism and structural information of the plaque (Bilen et al. 2018).

5.6.2 *In Vitro/Ex Vivo* Cell Imaging

In addition to their usage for *in vivo* imaging at the cellular and molecular levels, QDs have also been used widely as *in vitro* imaging agents (Table 5.1). Near-infrared QDs are an emerging novel class of fluorescent labels with strong tissue penetrability, sufficient electron density, excellent fluorescent stability and high fluorescent intensity. Such QDs possess the potential for *in vivo* imaging, early cancer diagnosis and high-resolution electron microscopy. Brunetti et al. constructed NT4 cancer-selective tetra-branched peptides conjugated with near-infrared QDs and functionalised them with amine-derived PEG. They also observed its promising role in imaging and targeting tumour cells in *in vivo* HT29 xenografted mice and *in vitro* HT29 cancer cells. The formulated near-infrared QDs with NT4 cancer-selective tetra-branched peptides were effective cancer theranostics as compared with the well-established high cancer selectivity. The PEG-coated QDs produced desired bioactivity with biocompatibility, stability, improved water solubility, tumour retention and reduced aggregation and systemic toxicity (Brunetti et al. 2018).

On the other side, nanometre-sized luminescent semiconductor QDs have also been involved as therapeutic and imaging agents in numerous diseases. Zhang et al. investigated cellular uptake, colloidal stability and toxicity of QDs in *in vivo*, *in vitro* and *ex vivo* environments of the brain. They observed that the behaviour of QDs is dependent on surface functionality and its treatment with cultured organotypic whole-hemisphere slices leads to increased metallothionein levels and dose-dependent toxicity. No change was obtained in the expression of mRNA in inflammatory cytokines or oxidative stress markers. PEG coating over the surface of QDs provided protection from aggregation in neurophysiological tissues and fluids. Notably, the brain microenvironment enables the development of QD-based imaging probes capable of targeting regions in the central nervous system and alters cellular interactions and localisation based on intended outcome (Zhang et al. 2019).

Geng et al. investigated the behaviour of clonal growth and analysed proliferation characteristics of varied cancer cells including SGC7901 human gastric cancer cell line, SW480 human colon cancer cell line and MCF 7 human breast cancer cell line. *In vitro* progression and tumour development were stimulated by cell clone formation assay. This was analysed using pan-CK and proliferating cell nuclear antigen Ki67, marked by different QD-conjugated probes. Parameters such as distribution in clone, Ki67 expression, discrete tendency, cell morphology and clone formation rate were investigated using QD-based molecular-targeted imaging. All three cell lines showed significant expression of Ki67 in clones and asymmetric growth behaviour. As a result, cell clone formation assay based on QD molecular imaging provided a novel method to understand the proliferative feature and morphological characteristics of cancer cells, providing an insight into tumour biology. This suggested that there is theoretical feasibility and technical possibility to develop a differentiation strategy which may allow for the control and coordination of cancer cells (Geng XF et al. 2016).

5.6.3 Tissue Imaging

Near-infrared fluorescence provides several advantages for *in vivo* and tissue imaging (Cassette et al. 2013). Optical imaging in preclinical and clinical settings provides enriched biological information, particularly when coupled with the targeted nanoparticles. Ryan et al. used clinical X-ray system to map the distribution of CdTe QD in mice to demonstrate excitation of X-rays of QDs emitting in near-infrared. They elicited near-infrared signals from the deep organ with short durable and tolerable radiations to permit *in vivo* applications. Notably, the application of keV X-rays to produce emission from tissue and QDs presents a novel bioimaging technology (Ryan et al. 2019).

5.6.4 Diagnostic Tool for Detection of Diseases

Tumour heterogeneity is one of the challenging and most important problems not only in understanding the mechanism of cancer but also in developing therapeutics to eradicate cancer cells. Liu et al. demonstrated the use of wavelength-resolved imaging and multiplexed QD–antibody conjugates for molecular mapping of human prostate cancer tissue. Multiplexed QD mapping provides morphological and molecular information for a clinical diagnostic application that is unavailable from traditional profiling and staining methods. They showed detection and characterisation of single and prostate gland cancer cells by using a panel of four protein biomarkers (α-methyl acyl-CoA racemase, p63, high molecular weight cytokeratin and E-cadherin). The results revealed the presence of tumour heterogeneity at the developmental, cellular and molecular

levels, which permits direct visualisation of prostate undergoing structural transitions up to malignancy of cells (Liu et al. 2010).

Further, Shi et al. developed a biocompatible multifunctional GQD-coated, high-luminescence magnetic nanoplatform for the selective separation and diagnosis of glypican-3 (GPC3)-expressed HepG2 liver circulating tumour cells from infected blood. Experimental results indicated that, because of the presence of a two-photon absorption cross section (40530 GM), an anti-GPC3 antibody-attached GOQD-coated magnetic nanoplatform could be incorporated as a two-photon luminescence platform for bright and selective imaging of HepG2 tumour cells in biological transparency window (960 nm). These results were evident from SK-BR-3 breast cancer cells and non-targeted GPC3(–) cells that showed two-photon imaging and are high selective for HepG2 hepatocellular carcinoma (HCC) tumour cells (Shi et al. 2015). Also, Morales-Narvaez et al. compared the potential of fluorescent dye Alexa 647 and CdSe/ZnS QDs as a reporter in sandwich immunocomplex microarray assay to detect apolipoprotein E. Although the performance of QDs varied as a function of excitation wavelength, they were proved as efficient reporters in microarrays. At 532 nm, QD microarray provided a limit of detection of ~62 pg/mL; however, at 633 nm, it provided a limit of detection of ~247 pg/mL. This was seven times more than that of ELISA and five times more than that of Alexa microarray. At last, human serum samples were also assessed, which gave high acceptability and sensitivity. Thus, the approach could be extended to the multiplexed detection of apolipoprotein and other Alzheimer-related biomarkers (Morales-Narváez et al. 2012).

5.6.5 Development of Diagnostic Test Systems

Samuel et al. studied the positively charged and negatively charged CdTe QDs' effect on human platelet functions in the absence or presence of plasma. They investigated interactions of QDs with platelet using transmission electron microscopy, atomic force microscopy and immunofluorescence. Also, the QD–platelet effects were screened using gelatin zymography, flow cytometry, quartz crystal microbalance with dissipation, and light aggregometry. They showed that binding of QDs with platelet plasma membrane was due to matrix metalloproteinase-2 release and upregulation of glycoprotein IIB/IIIa and P-selectin receptors. Further, the mechanism of the functional response of platelets to ultra-small QDs was unravelled for the first time. They reported that QDs can stimulate platelet aggregation in both underflow and no-flow conditions (Samuel et al. 2015). Kim et al. developed a clinical validation of QD barcode-based technology for diagnosing hepatitis B virus. Also, involvement of multiplexed QD barcode for the detection of multiple regions of the viral genome was reported to improve clinical sensitivity (Kim et al. 2016). Zhang et al. developed a sensitive and simple method for the detection of infectious disease markers that conjugate the dot-blot immunoassay with reporter as CdSe/ZnSe/ZnS core/shell/shell QD nanobeads prepared using oil-in-water emulsion evaporation technique. They detected proteins in a step test by developing QD nanobeads as a signal indicator, as low as 78 pg hepatitis surface antigen (Zhang et al. 2014).

5.6.6 Biosensors/Biomarkers for Detection of Mutation, Multiplexed Target and miRNA Detection

Since the last few decades, one of the greatest achievements in nanomaterials has been the development of biosensors. Biosensors are devices containing biological sensing elements either integrated or connected in transducers. After recognition of specific molecules in the body on the basis of structure including receptor hormone, enzyme–substrate, antibody and antigen, biosensors exhibit their mechanism of action (Kricka 1988; Buch and Rechnitz 1989; Zhang et al. 2009). To produce highly efficient biosensors, the substrate selected for sensing material dispersion is a prerequisite. Different types of nanomaterials, including golden nanoparticles, carbon nanotubes, magnetic nanoparticles and QDs, can be applied as biosensors. Involving QDs with their unique properties has provided novel strategies for quantification and identification of biologically relevant molecules (Matea et al. 2017).

Chan and Nie were the first to publish reports of ZnS-CdSe QDs for ultrasensitive non-isotropic detection (Chan and Nie 1998). Johri-Ahar et al. presented a novel nanostructured immunosensor for early detection of cancer antigen 125 (CA-125) serum biomarker in patients with ovarian cancer. The immunosensor was composed of gold electrode-modified mercaptopropionic acid (MPA) and conjugated with anti-CA-125 monoclonal antibody (mAb), CdSe QDs and silica-coated gold nanoparticles (AuNP-SiO_2). This conjugation resulted in the sensitive detection of CA-125 down to 0.0016 u/mL. They characterised the engineered MPA-AuNP-SiO2-QD-mAb immunosensor using different spectral techniques (Johari-Ahar et al. 2015). Yang et al. used spectroscopic fluorescence techniques to propose a sensitive sensor for probing the interaction of proteins with clofazimine (an effective drug against multidrug-resistant breast cancer and tuberculosis). They developed CdZnSeS/ZnS alloyed core/thick-shell QDs as energy donors Förster resonance energy transfer (FRET) applications. These QDs were further coated with multifunctional polymers containing dihydrolipoic acid through a direct ligand exchange method and functionalised with cyanine-3-labelled human serum albumin (Yang et al. 2016).

Andreadou et al. established a Leishmania-specific surface antigen detection method based on a combination of CdSe QDs and magnetic beads with a low limit of detection (3125 ng/μL) and high specificity for Leishmania DNA. Thus, the principle behind the method is that the detection was performed by QDs and analytes were isolated from solution with the help of magnetic beads (Andreadou et al. 2016). In another study, core/multi-shell QDs (CdSe@ZnS@

CdS@ZnS) produce 100% quantum yield with photoluminescence as high as obtained by layer-by-layer deposition. The coating was performed layer by layer, where each layer deposited onto CdSe core had some specific work. The first ZnS shell acted as a potential barrier for electron–hole pairs, the ZnS shell acted as an outer shell or potential barrier, and the CdSe shell acted as a separator between two ZnS shells. They characterised two-photon properties of QDs with varied sizes. Thus, the obtained large two-photon excitation acts cross-sectionally and makes QDs an efficient photoluminescent material for multiphoton microscopy (Linkov et al. 2016). Geng et al. proposed CdSe-ZnS-conjugated haem nanoprobes in an *in vivo* and *in vitro* experimental set-up to investigate the haem iron absorption complex. Their results presented that such conjugation is suitable for tracing ham iron absorption by both endocytosis and active transport haemoglobin carrier protein-1 pathways involved in haem uptake in Caco-2 cells (Geng L et al. 2016).

5.6.7 Drug Delivery/Carrier for Treatment

Over the last decade, researchers have paid great attention to the multimodal drug delivery system. Due to unique physicochemical properties, QDs are considered as an efficient tool in theranostic applications also (Zayed et al. 2019). The term theranostics can be defined as ongoing efforts to develop individualised and more specific therapies for various diseases and to combine therapeutics and diagnostics into a single agent. Advanced drug delivery systems have been designed as strategies to resolve many complications associated with conventional formulations and provide site-specific targeted delivery of a drug (Singh and Bajwa 2016). Further strategies to increase biocompatibility and reduce toxicity have also been developed through hybridisation with lipids, polysaccharides, protein or polymers, offering tumour targeting and enhanced bioavailability (Zayed et al. 2019).

5.6.7.1 Ocular Diseases

QDs have always been suitable for multi- and single-colour bioimaging of biomolecules. QDs with near-infrared emission have been developed for *in vivo* imaging due to their absorption and scattering ability of incident light, yielding good optical signals and tissue penetration. In ophthalmology, an autofluorescence may be caused due to visible light from ocular structures leading to a reduction in the contrast of ocular fluorescence. However, QDs have the ability to offer both near-infrared and visible emission of the electromagnetic spectrum without disturbing autofluorescence and also offer a range of applications such as labelling and bioimaging in ophthalmology (Sarwat et al. 2019). Inefficient penetration across the plasma membrane has always hampered the delivery of gene, therapeutic molecule and drug to ocular tissues. Johnson et al. used a novel peptide with protein transduction properties for ocular delivery of small and large molecules across plasma membrane *in vivo* and *in vitro*. The peptide was able to enter the cell in a temperature-dependent manner within 5 min. Also, they reported that the peptide has a tendency to compact and deliver plasmid DNA. Small interfering RNA duplexes to cell using the peptide allowed silencing and achieving transgene expression in more than 50% human embryonic retinoblasts. They evidenced that the peptide for ocular delivery entered neural retina and localised to the ganglionic cell, photoreceptors and retinal pigment epithelium.

Moreover, peptides were also able to enter dura of optic nerve, choroid, sclera and corneal epithelium via topical application. The peptide for ocular delivery functions as bacteriostatic, which enables it to be a carrier of molecules to post-mitotic neural ocular tissue (Johnson et al. 2008). Further, Olsan et al. administered single intravitreal injection of biotin-conjugated QDs composed of Cd/Se coated with a thin layer of ZnS in the Royal Chemical Society rat model of progressive photoreceptor degeneration. Over weeks of post-procedure, they observed a gradual reduction in the amplitude of electroretinogram recording. They reported the effectiveness of intravitreal injection of photoactive QDs as a technology in progressive retinal degenerations followed by increased retinal electrical activity (Olson et al. 2012). In line with this, Olsan et al. also performed another experiment where they administered a single intravenous injection of biotin-conjugated photoactive QDs in the Royal Chemical Society rat model of progressive photoreceptor degeneration. Over 6 weeks of post-procedure, they observed a gradual reduction in the amplitude of electroretinogram recording. They used photoactive QDs as carriers to deliver localised electrical stimulation in retinal degeneration. The technology also possesses a tendency to overcome limitations associated with presently available treatment for blinding retinal disease. First, QD was able to deliver more precisely at the cellular level. Second, the ability of QD to conjugate allows precise targeting of different cells within retina. Third, this strategy can be employed as preventive rather than reversing a loss in pronounced photoreceptor degeneration. Fourth, therapy can be delivered with a needle rather than significant eye surgery (Olson and Mandava 2014).

5.6.7.2 Cardiovascular Diseases

Sun et al. designed trifunctional simian virus (SV40)-based nanoparticles for *in vivo* targeting and imaging of atherosclerotic plaque. Further, they encapsulated near-infrared QDs in nanoparticles and also incorporated Hirulog, an anticoagulant drug. They imaged fluorescent atherosclerotic plaque non-invasively in live ApoE-deficient mice. Targeted SV40-based nanoparticles were also able to deliver Hirulog to an atherosclerotic plaque region efficiently. The SV40-based nanoparticles encapsulating QDs showed remarkable optical properties for *in vivo* imaging. Also, early, developmental and late stages of atherosclerosis were quickly targeted and imaged in a live animal using targeting peptides for fibrin, macrophage and vascular cell adhesion molecule-1. Thus, a multifunctional

and multivalent SV40-based nanoparticle was developed suitable for drug delivery, molecular targeting and *in vivo* imaging (Sun et al. 2016).

5.6.7.3 Neurological Disorders

Crossing the blood–brain barrier for the delivery of drugs and active agents from blood to the central nervous system is one of the challenging issues faced by scientists. The development of functionalised bioconjugated QDs to address this challenge for both treatment and diagnosis of brain diseases, such as HIV-associated encephalopathy or Alzheimer's disease, had been investigated in nanomedicine (Xu et al. 2013).

Medina et al. designed a novel approach for barcoding nanoparticles composed of poly(lactic-co-glycolic acid) (PLGA) with spectrally defined QDs to allow direct fluorescent detection of nanoparticle fate with subcellular resolution. Also, the biophysical properties of nanoparticles or their interaction with other cells are unaffected by QD labelling. *In vivo* imaging allowed simultaneous visualisation of the interaction of bEnd.3 cells with the targeted nanoparticle, confirming that surface modification with TAT, a CPP, increases their biophysical association with cell surfaces. Further, this modification with the CPP also facilitates brain-specific delivery specifically to brain vasculature along with tracking and imaging (Medina et al. 2017).

Paris-Robidas et al. conjugated mAbs (Ri7) with QDs targeting murine transferrin receptor. They were the first to confirm specific transferrin receptor-mediated endocytosis of QD–Ri7 in bEnd5 and N2A cells. As compared to control, intravenously injected Ri7-QD showed a fourfold higher volume of distribution in brain tissues. Most QDs within brain capillary endothelial cells were seen in small vesicles, with a small portion in multivesicular bodies and tubular structures. Parenchymal penetration of QD–Ri7 was shallow and even comparable to IgG. Thus, results showed that the QD–Ri7 complex undergoes endocytosis by brain capillary endothelial cells if administered systemically (Paris-Robidas et al. 2016).

5.6.7.4 Hepatic Diseases

Hunt et al. showed targeted delivery of Ag_2S QDs to hepatocytes *in vitro* and *in vivo* or liver sinusoidal endothelial cells (LSECs) and rapid absorption across small intestine after oral administration. The QDs were radiolabelled with ^{14}C-metformin, a fluorescent tag or 3H-oleic acid within a drug binding site. They also included three biopolymer shell coatings, namely heparin, gelatin and formaldehyde-treated serum albumin (FSA). The passage across the small intestine into mesenteric veins was mediated via micropinocytosis and clathrin endocytosis. Two hours post-ingestion, the bioavailability of ^{14}C-metformin was increased fivefold by conjugation with QD-FSA, whereas uptake of metformin to LSECs was improved 50-fold by using such QDs. Endocytosis of QDs by SK-Hep1 cells was via QD-conjugated caveolae and clathrin-mediated pathways taken up into lysosomes (Hunt et al. 2020).

Shi et al. produced HCC-targeted delivery vehicles by covalently coupling 5-fluorouracil acetic acid (FUA) and folic acid on the surface of ZnCdSe/ZnS QDs (Folic acid-QDs-FUA). The prepared complex was characterised using different spectral techniques. The drug-loading content, zeta potential and average particle size were 36.85% ± 1.61%, –13.3 mV and 220.28 nm, respectively. Folic acid-QDs-FUA showed reduced cytotoxicity and targeted the HCC (HepG2 and SMMC-7721) more easily. *In vivo* experiments showed that mice treated with Folic acid-QDs-FUA produced superior tumour suppression. Thus, Folic acid-QDs-FUA can be used for improving the efficacy of 5-FU and tumour targeting with limited toxicity (Shi et al. 2018).

5.6.7.5 Antibiotic-Resistant Infection

Sarkar et al. prepared highly luminescent carbon QDs from aloe vera leaves using the carbonisation pathway. The prepared QDs were characterised through different spectral techniques, and functional carbon QDs were screened to be non-cytotoxic. Also, a cytotoxicity study of prepared carbon QDs was performed in HepG2, A549 and HeLa cell lines. The high aqueous dispersibility, less cytotoxicity and biocompatibility of synthesised carbon QDs were aimed to design carbon QDs tailored calcium alginate (CA) hydrogen films with goal to controlled delivery of vancomycin in the gastrointestinal tract. The drug-loading capacity of CA/carbon QDs was increased to 89% as compared to CA/carbon QDs with β-cyclodextrin (β-CD), which increased to 96%. Thus, at pH 1.5, a lower release rate (56% in 120 h) and high drug-loading capacity of CA/carbon QDs with β-CD can be utilised for drug delivery (Sarkar et al. 2017).

Wansapura et al. developed chitin–CdTe QD hybrid films with an antibacterial effect against Pseudomonas aeruginosa and Staphylococcus aureus by combining CdTe QDs with chitin using the facile aqueous synthesis route. They characterised chitin–CdTe QD using X-ray diffraction analysis, thermogravimetric analysis, Fourier transform infrared spectroscopy, energy-dispersive X-ray spectroscopy and high-resolution field emission scanning electron microscopy. They also reported that an efficient antibacterial activity was shown by chitin–CdTe QD films against Gram-negative and Gram-positive bacteria. Conclusively, they evidenced that chitin–CdTe QD films might be desirable antibacterial agents for a variety of biomedical applications, including implants, ophthalmology, packaging, drug delivery systems, burn treatments and wound dressings (Wansapura et al. 2017).

5.6.7.6 Tumours

At times, the direct intracellular delivery of peptides or proteins has remained a challenge due to the bioavailability barrier across the membrane. Several pharmacological agents were discovered using the traditional approach to modulate protein functions and deliver across the cell. However, these specific molecules and drugs possess some limitations, such as poor tissue distribution, toxicity, unwanted side effects and target specificity. Likewise, over

the past decades, tremendous advances have been made for the development of molecular techniques for gene delivery and protein expressions, but they have been of surprisingly little benefit for the management of genetic disorders. Apart from these, *in vivo* gene therapy approaches based on adenoviral vectors are also associated with toxicity and lack of specificity (Wadia and Dowdy 2005). Based on cell-penetrating motif of an anti-cancer peptide, i.e. buforin IIb, Lim et al. developed CPPs with cancer cell specificity. They found a 17-amino acid peptide (BR2) to be a cancer-specific derivative among all derivatives, without any cytotoxicity. BR2 entered the cell through lipid-mediated macropinocytosis after specifically targeting cancerous cells via interactions with gangliosides. Besides, BR2 was reported to translocate efficiently as compared to the CPP TAT (49-57). BR2 fused with single-chain variable fragment (scFv) directed towards K-ras-mutated HCT116 cells to demonstrate ability of BR2 as specific cancer cell carrier. It was reported that BR2-fused scFv shows a higher degree of apoptosis when compared with TAT-fused scFv. They concluded that the CPP BR2 possesses great potential to be used as a drug delivery carrier with cancer cell specificity (Lim et al. 2013).

Iannazzo et al. synthesised graphene QD (GQD)-based biocompatible and fluorescent nanovector for targeted delivery of benzofuran structure (BFG)-derived anti-cancer drug bearing targeting ligand riboflavin. The highly water-dispersible nanoparticles were covalently linked to the anti-cancer drug via cleavage PEG linker, while the ligand riboflavin (RF) was conjugated with graphene QD by π–π interaction using a pyrene (Pyr) linker. They showed that the designed drug delivery system (GQD-PEG-BFG-Pyr-RF) has less cytotoxic effect, and subsequently, its impact on three cancer cell lines was compared with the effect produced by the anti-cancer drug (GQD-Pyr-RF) which was similar to nanovector lacking targeted riboflavin ligand (GQD-PEG-BFG) (Iannazzo et al. 2019).

Nifontova et al. screened the cytotoxicity of QD-encoded microcapsules and validated an approach for the activation of microcapsule's surface for functionalisation with trastuzumab. Trastuzumab is a clinically available humanised mAb for the treatment of breast cancer-targeting human epidermal growth factor receptor 2 (HER2). They reported that polymer shell-encapsulated QDs synthesised using the layer-by-layer deposition method produce a highly fluorescent polyelectrolyte microcapsule with biocompatibility and homogenous size distribution. Activation of carbodiimide surface evidenced that QD-encapsulated microcapsules show optical characteristics and optimal dispersion before conjugation with antibody. Moreover, the conjugate of mAb targeting HER2 and microcapsule provided specific and sensitive antibody-mediated binding of microcapsule with living cancer cells (Nifontova et al. 2019).

Li et al. formulated a smart nanosystem for reducing side effects and improving the therapeutic efficiency of mitoxantrone (MTN) by cross-linking carboxylated GQDs (cGQDs) with NH2-PEG-NH2 and folic acid modification. The novel drug delivery system showed a remarkable drug-loading capacity (40.1%) and entrapment efficiency (97.5%). Morphological studies, i.e. cell imaging, showed that the nanosystem enters human cervical cells via a micropinocytosis-dependent pathway. Further, the nanodelivery system showed low systemic toxicity, improved anti-tumour ability and targeting capacity in a live animal without having any systemic adverse effects (Li et al. 2019).

Khodadadei et al. synthesised blue-fluorescent nitrogen-doped GQDs using the hydrothermal method with urea as a nitrogen source and citric acid as a carbon source. Fourier transform infrared resonance spectroscopy confirmed the existence of nitrogen-doped GQDs. Further, methotrexate (MTX), an anti-cancer drug, was loaded within nitrogen-doped GQDs to prepare MTX–nitrogen-doped GQDs as a delivery system. Successful loading of MTX in nitrogen-doped GQDs was indicated by the presence of a strong π-π stacking interaction between nitrogen-doped GQDs and MTX as confirmed by UV spectroscopy and Fourier transform infrared resonance spectroscopy. Also, the *in vitro* cytotoxicity study on MCF-7 breast cancer cell line showed that MTX–nitrogen-doped GQDs are toxic than drug-free nitrogen-doped GQD nanocarriers (Khodadadei et al. 2017).

Olerile et al. screened the potential of a co-loaded [QDs (CdTe/CdS/ZnS) and paclitaxel] nanostructured lipid carriers (NLC) as a parenteral multifunctional delivery system. The co-loaded NLC was formed by the low-temperature solidification and emulsion evaporation methods using soya phosphatidylcholine, oleic acid and glyceryl monostearate as lipid matrix. In characterising co-loaded NLC, the zeta potential, polydispersity index and particle size were found, -0.22 ± 0.03 mV, 0.17 ± 0.04 mV and 115.93 ± 1.61 nm, respectively. A higher drug-loading capacity of $4.68\% \pm 0.04\%$ and entrapment efficiency of $80.70\% \pm 2.11\%$ were recorded with a spheroid-like shape and smooth surface. The tumour growth inhibition rate and IC_{50} were found to be 77.85% and 1.05 ± 0.58 M, respectively. The *ex vivo* and *in vivo* results of co-loaded NLC indicated its capability to target and detect H22 tumour. Thus, the co-loaded NLC formulation was qualified as a parenteral drug delivery system for cancer theragnostic (Olerile et al. 2017).

Li et al. for the first time explored mitochondria as a delivery system of doxorubicin (Dox) and carbon QDs for *in vivo* imaging. To achieve *in vivo* imaging, they prepared near-fluorescent carbon QD (DyLight 680) and loaded it in the mitochondria (Mito-CQD-Dy680). As a carrier, mitochondrion was found to be compatible with carbon QD and also preserves the optical properties of carbon QD. Moreover, the system also improves the biodistribution of carbon QD and increases its retention time after intravenous injection. An enhanced therapeutic effect was shown by mitochondrion loaded with Dox as compared to free Dox. Thus, the mitochondria-based aircraft system possesses high potential for drug delivery and bioimaging against cancer and other diseases (Li et al. 2018).

5.6.7.7 Renal Diseases

Renal disease has become a prevalent problem to public health for which the application of kidney-related drug delivery system has profound transformative potential. Such drug delivery system enhances the efficacy and reduces undesired side effects of the potent drug for treating renal diseases (Liu et al. 2019).

5.6.7.8 Others

Omidi et al. produced vascular endothelial growth factor (VEGF)-loaded PLGA carbon QD microspheres using microfluidic platforms. VEGF was PEGylated to prevent structural stability and protein functional stability during the encapsulation process. The produced microsphere was highly monodispersed and intact. The loading efficiency of PEGylated VEGF in microparticles varied from 51% to 69%, and >90% of PEGylated VEGF was even released within 28 days, which was monitored by carbon QDs (Omidi et al. 2019).

5.6.8 Cell Labelling

The optical properties of QDs, precisely the wavelength of their fluorescence, depend on their size. Typically, such small size provides no direct molecular or structural observations from microscopy and needs to be labelled with a marker to be observed. It is well known that fluorescent labelling is one of the modest techniques involved in cell biology. Since QDs have unique and constant optical properties, they were utilised for the purpose of cell labelling. Interestingly, QDs have the ability to tag intracellular and multi-inner components concurrently in living cells for varied time intervals. They can be attached to a molecule like an antibody that will bind the target, or they can bind the target to be visualised. Different coloured QDs have the ability to label varied cellular components, which can be easily visualised *in vivo* or with fluorescence microscopy (Parak et al. 2005; Bajwa et al. 2016). For instance, ganglion was labelled by the conjugate of biotinylated cholera toxin B with QD–avidin for animal bioimaging, lignin and cellulose were labelled with CdSe QDs for plant bioimaging (Cheki et al. 2013), and anti-E. coli 0157-coated and streptavidin-coated were used for measuring bacterial cell in prokaryotic imaging and (Bajwa et al. 2016).

Further, the light stability of QD markers makes long-term tracking of biological molecules possible, using the labelling technique. Monitoring interactions among and within the living cells as they differentiate after growing is crucial for understanding the development of organisms. In reference to this, fluorescence microscopy has been the most widely used approach for non-invasive and high-resolution *in vivo* imaging and organic fluorophores have been the commonly used tag in fluorescence microscopy but with few limitations. Fluorescent QDs an inorganic fluorescent nanocrystal emerged to overcome the barriers and function as useful alternative for multicolour imaging. Jaiswal et al. used the mixed-surface self-assembly approach and positively charged adapters to prepare protein-conjugated QDs coated with negatively charged dihydroxylipoic acid. For instance, naturally charged avidin protein allows stable conjugation of QDs to antibodies or ligands that can be biotinylated, whereas the involvement of protein fused to oligohistidine peptide or positively charged leucine zipper peptide obviates the need for biotinylating the target molecule (Jaiswal et al. 2004).

Valley et al. involved two-coloured single QD tracking to analyse kinetics of receptor dimerisation on living. They showed that mutants are capable of forming stable ligand-independent dimers as compared to wild-type epidermal growth factor receptor (EGFR/erbB1/Her1). Several EGFR mutants have proved that ligand-independent signals are commonly available in non-small-cell lung cancers, including exon 19 deletion and kinase domain mutations L858R. Measurements of live-cell FRET showed that mutations of L858R kinase modify the ectodomain structure such that the unliganded mutant EGFR adopts a dimerisation-competent and extends confirmation. Such results support the model considering that dysregulated activity of non-small-cell lung cancer-associated kinase mutants is driven by the interaction of both extracellular domains and kinase that leads to dimerisation (Valley et al. 2015).

Despite having attractive properties such as photostability, brightness and the ability to excite different QDs with single wavelength over fluorescent proteins and dyes, only a few QD conjugates are commercially available for labelling cellular targets. Francis et al. determined the specificity of commercially available QD625 conjugated with a secondary antibody against primary IgG antibody. Simultaneously, the antigen was labelled with fluorescent dye coupled to the secondary antibody and QD 525-conjugated secondary antibody. They observed that fluorescent dye-coupled secondary antibody labelled all intended targeted proteins, but the QD-conjugated secondary antibody, specifically anti-tubulin QD625 conjugate, was able to bind quickly to some of the cytosol-based protein targets only. This labelling corresponded to steric hindrance associated with the size of the QD-conjugated secondary antibody (Francis et al. 2017).

Tracking human mesenchymal stem cells (MSCs) in living cells has remained a critical component of the evaluation of efficacy and safety of therapeutic cell products. Thus, the cell needs to be labelled with agents to allow visualisation of migration of MSCs. Kundrotas et al. designed a study to evaluate the cytotoxicity, uptake dynamics and extracellular and subcellular distribution of non-targeted carboxylated QDs in the bone marrow. They revealed that no negative impact is produced by QD on the viability of MSCs throughout the experimental period in cells. However, the presence of lipid droplets was observed in some MSCs. At low cell growing densities, QDs distribute within the MSC cytoplasm already after 1 h of incubation, reaching saturation after 6 h. Also, QDs prominently mark MSC long filopodia-like structures attaching neighbouring cells, whereas at high-cell-density cultivation, QD distribution was prominent in the extracellular matrix of MSCs.

Interestingly, the average photoluminescence time of distribution of QDs in the extracellular matrix was more than a lifetime of QD entrapped in endosomes. In conclusion, they stated that carboxylated QDs could be used as an effective and non-specific dye for labelling bone marrow MSCs (Kundrotas et al. 2019).

5.7 CONCLUSION

In recent years, QDs have attracted fascinating attention as a promising and most valuable candidate in areas of imaging, sensor, labelling and drug delivery. Their favourable characteristics such as good biocompatibility, cost-effectiveness and low toxicity emphasise their use as a theranostic agent for cancer and as a drug delivering agent in various diseases affecting systems such as renal, hepatic, neurological, cardiovascular and ocular. Further, the advancements in the field of QDs have reduced their cytotoxicity, expanded the scope of their biomedical applications and rendered QDs as the crucial device in the research of cellular processes such as receptor trafficking, uptake and intracellular delivery. Also, a part has shown a significant breakthrough in the field for antibiotic-resistant infections, microbial infections and detection of antigens. Owing to the initial success of QDs and their conjugates employed in biomedical applications, it is convincing that future research will continue to focus on the identification of various tumours with minimal side effects and evaluate the mechanisms related to drug and disease. Different ongoing research on QDs targets achieving a selective approach for labelling, better fluorescence and higher biosafety. Also, vigorous research is focusing on studying the effects of interference of QDs with normal physiology. To see the realistic translation of QDs into clinical applications, several issues still need to be addressed, such as environmental impact, synthesis protocol scalability, body clearance and overall toxicity.

REFERENCES

Amaral PE, Hall Jr DC, Pai R, Król JE, Kalra V, Ehrlich GD, Ji H-F. 2020. Fibrous phosphorus quantum dots for cell imaging. *ACS Applied Nano Materials*. 3(1): 752–759.

Andreadou M, Liandris E, Gazouli M, Mataragka A, Tachtsidis I, Goutas N, Vlachodimitropoulos D, Ikonomopoulos J. 2016. Detection of Leishmania-specific DNA and surface antigens using a combination of functionalized magnetic beads and cadmium selenite quantum dots. *Journal of Microbiological Methods*. 123:62–67.

Aswathi M, Ajitha A, Akhina H, Lovely M, Thomas S. 2018. Quantum dots: a promising tool for biomedical application. *JSM Nanotechnology & Nanomedicine*. 6:1066.

Bajwa N, Mehra NK, Jain K, Jain NK. 2016. Pharmaceutical and biomedical applications of quantum dots. *Artificial Cells, Nanomedicine, and Biotechnology*. 44(3):758–768.

Ballou B, Ernst LA, Andreko S, Harper T, Fitzpatrick JA, Waggoner AS, Bruchez MP. 2007. Sentinel lymph node imaging using quantum dots in mouse tumor models. *Bioconjugate Chemistry*. 18(2):389–396.

Bannai H, Inoue T, Hirose M, Niwa F, Mikoshiba K. 2020. Synaptic function and neuropathological disease revealed by quantum dot-single-particle tracking. In: Yamamoto N, Okada Y, editors. *Single Molecule Microscopy in Neurobiology*. Springer: New York; pp. 131–155.

Bera D, Qian L, Tseng T-K, Holloway PH. 2010. Quantum dots and their multimodal applications: a review. *Materials*. 3(4):2260–2345.

Bilen B, Gokbulut B, Kafa U, Heves E, Inci MN, Unlu MB. 2018. Scanning acoustic microscopy and time-resolved fluorescence spectroscopy for characterization of atherosclerotic plaques. *Scientific Reports*. 8(1):1–11.

Brunetti J, Riolo G, Gentile M, Bernini A, Paccagnini E, Falciani C, Lozzi L, Scali S, Depau L, Pini A. 2018. Near-infrared quantum dots labelled with a tumor selective tetrabranched peptide for *in vivo* imaging. *Journal of Nanobiotechnology*. 16(1):1–10.

Buch R, Rechnitz G. 1989. Intact chemoreceptor-based biosensors: responses and analytical limits. *Biosensors*. 4(4):215–230.

Caglar M, Pandya R, Xiao J, Foster SK, Divitini G, Chen RY, Greenham NC, Franze K, Rao A, Keyser UF. 2019. All-optical detection of neuronal membrane depolarization in live cells using colloidal quantum dots. *Nano Letters*. 19(12):8539–8549.

Cassette E, Helle M, Bezdetnaya L, Marchal F, Dubertret B, Pons T. 2013. Design of new quantum dot materials for deep tissue infrared imaging. *Advanced Drug Delivery Reviews*. 65(5):719–731.

Chan WC, Nie S. 1998. Quantum dot bioconjugates for ultrasensitive nonisotopic detection. *Science*. 281(5385):2016–2018.

Cheki M, Moslehi M, Assadi M. 2013. Marvelous applications of quantum dots. *European Review for Medical and Pharmacological Sciences*. 17(9):1141–1148.

Claro MS, Levy I, Gangopadhyay A, Smith DJ, Tamargo MC. 2019. Self-assembled Bismuth Selenide (Bi_2 Se_3) quantum dots grown by molecular beam epitaxy. *Scientific Reports*. 9(1):1–7.

Efros AL, Delehanty JB, Huston AL, Medintz IL, Barbic M, Harris TD. 2018. Evaluating the potential of using quantum dots for monitoring electrical signals in neurons. *Nature Nanotechnology*. 13(4):278–288.

Efros AL, Nesbitt DJ. 2016. Origin and control of blinking in quantum dots. *Nature Nanotechnology*. 11(8):661.

Ehlers MD, Heine M, Groc L, Lee M-C, Choquet D. 2007. Diffusional trapping of GluR1 AMPA receptors by input-specific synaptic activity. *Neuron*. 54(3):447–460.

Francis JE, Mason D, Lévy R. 2017. Evaluation of quantum dot conjugated antibodies for immunofluorescent labelling of cellular targets. *Beilstein Journal of Nanotechnology*. 8(1):1238–1249.

Geng L, Duan X, Wang Y, Zhao Y, Gao G, Liu D, Chang Y-Z, Yu P. 2016. Quantum dots-hemin: preparation and application in the absorption of heme iron. *Nanomedicine: Nanotechnology, Biology and Medicine*. 12(7):1747–1755.

Geng XF, Fang M, Liu SP, Li Y. 2016. Quantum dot-based molecular imaging of cancer cell growth using a clone formation assay. *Molecular Medicine Reports*. 14(4):3007–3012.

Horoz S, Lu L, Dai Q, Chen J, Yakami B, Pikal J, Wang W, Tang J. 2012. CdSe quantum dots synthesized by laser ablation in water and their photovoltaic applications. *Applied Physics Letters*. 101(22):223902.

Hunt NJ, Lockwood GP, Le Couteur FH, McCourt PA, Singla N, Kang SWS, Burgess A, Kuncic Z, Le Couteur DG, Cogger VC. 2020. Rapid intestinal uptake and targeted delivery to the liver endothelium using orally administered silver sulfide quantum dots. *ACS Nano.* 14(2): 1492–1507.

Iannazzo D, Pistone A, Celesti C, Triolo C, Patané S, Giofré SV, Romeo R, Ziccarelli I, Mancuso R, Gabriele B. 2019. A smart nanovector for cancer targeted drug delivery based on graphene quantum dots. *Nanomaterials*. 9(2):282.

Jaiswal JK, Goldman ER, Mattoussi H, Simon SM. 2004. Use of quantum dots for live cell imaging. *Nature Methods*. 1(1):73–78.

Jayagopal A, Su YR, Blakemore JL, Linton MF, Fazio S, Haselton FR. 2009. Quantum dot mediated imaging of atherosclerosis. *Nanotechnology.* 20(16):165102.

Jie G, Zhao Y, Niu S. 2013. Amplified electrochemiluminescence detection of cancer cells using a new bifunctional quantum dot as signal probe. *Biosensors and Bioelectronics*. 50:368–372.

Jin S, Hu Y, Gu Z, Liu L, Wu H-C. 2011. Application of quantum dots in biological imaging. *Journal of Nanomaterials*. 2011, Article ID 834139.

Johari-Ahar M, Rashidi M, Barar J, Aghaie M, Mohammadnejad D, Ramazani A, Karami P, Coukos G, Omidi Y. 2015. An ultra-sensitive impedimetric immunosensor for detection of the serum oncomarker CA-125 in ovarian cancer patients. *Nanoscale*. 7(8):3768–3779.

Johnson LN, Cashman SM, Kumar-Singh R. 2008. Cell-penetrating peptide for enhanced delivery of nucleic acids and drugs to ocular tissues including retina and cornea. *Molecular Therapy.* 16(1):107–114.

Khodadadei F, Safarian S, Ghanbari N. 2017. Methotrexate-loaded nitrogen-doped graphene quantum dots nanocarriers as an efficient anticancer drug delivery system. *Materials Science and Engineering: C*. 79:280–285.

Kim J, Biondi MJ, Feld JJ, Chan WC. 2016. Clinical validation of quantum dot barcode diagnostic technology. *ACS Nano*. 10(4):4742–4753.

Kricka L. 1988. Molecular and ionic recognition by biological systems. In: Edmonds TE, editor. *Chemical Sensors*. Blackie and Sons: Glasgow; pp. 3–14.

Kundrotas G, Karabanovas V, Pleckaitis M, Juraleviciute M, Steponkiene S, Gudleviciene Z, Rotomskis R. 2019. Uptake and distribution of carboxylated quantum dots in human mesenchymal stem cells: cell growing density matters. *Journal of nanobiotechnology*. 17(1):39.

Lalu L, Tambe V, Pradhan D, Nayak K, Bagchi S, Maheshwari R, Kalia K, Tekade RK. 2017. Novel nanosystems for the treatment of ocular inflammation: current paradigms and future research directions. *Journal of Controlled Release*. 268:19–39.

Lee S, Jung KJ, Jung HS, Chang S. 2012. Dynamics of multiple trafficking behaviors of individual synaptic vesicles revealed by quantum-dot based presynaptic probe. *PLoS One*. 7(5):e38045.

Li W-Q, Wang Z, Hao S, Sun L, Nisic M, Cheng G, Zhu C, Wan Y, Ha L, Zheng S-Y. 2018. Mitochondria-based aircraft carrier enhances *in vivo* imaging of carbon quantum dots and delivery of anticancer drug. *Nanoscale*. 10(8):3744–3752.

Li Z, Fan J, Tong C, Zhou H, Wang W, Li B, Liu B, Wang W. 2019. A smart drug-delivery nanosystem based on carboxylated graphene quantum dots for tumor-targeted chemotherapy. *Nanomedicine*. 14(15):2011–2025.

Lim KJ, Sung BH, Shin JR, Lee YW, Yang KS, Kim SC. 2013. A cancer specific cell-penetrating peptide, BR2, for the efficient delivery of an scFv into cancer cells. *PloS one*. 8(6):e66084.

Lim YT, Kim S, Nakayama A, Stott NE, Bawendi MG, Frangioni JV. 2003. Selection of quantum dot wavelengths for biomedical assays and imaging. *Molecular Imaging*. 2(1):15353500200302163.

Linkov P, Krivenkov V, Nabiev I, Samokhvalov P. 2016. High quantum yield CdSe/ZnS/CdS/ZnS multishell quantum dots for biosensing and optoelectronic applications. *Materials Today: Proceedings*. 3(2):104–108.

Liu BR, Winiarz JG, Moon J-S, Lo S-Y, Huang Y-W, Aronstam RS, Lee H-J. 2013. Synthesis, characterization and applications of carboxylated and polyethylene-glycolated bifunctionalized InP/ZnS quantum dots in cellular internalization mediated by cell-penetrating peptides. *Colloids and Surfaces B: Biointerfaces*. 111:162–170.

Liu CP, Hu Y, Lin JC, Fu HL, Lim LY, Yuan ZX. 2019. Targeting strategies for drug delivery to the kidney: from renal glomeruli to tubules. *Medicinal Research Reviews*. 39(2):561–578.

Liu J, Lau SK, Varma VA, Moffitt RA, Caldwell M, Liu T, Young AN, Petros JA, Osunkoya AO, Krogstad T. 2010. Molecular mapping of tumor heterogeneity on clinical tissue specimens with multiplexed quantum dots. *ACS Nano*. 4(5):2755–2765.

Liu S, Su X. 2014. The synthesis and application of I–III–VI type quantum dots. *RSC Advances*. 4(82):43415–43428.

Ma Y, Wang M, Li W, Zhang Z, Zhang X, Tan T, Zhang X-E, Cui Z. 2017. Live cell imaging of single genomic loci with quantum dot-labeled TALEs. *Nature Communications*. 8(1):1–8.

Maheshwari R, Chourasiya Y, Bandopadhyay S, Katiyar PK, Sharma P, Deb PK, Tekade RK. 2018. Chapter 1 – Levels of solid state properties: role of different levels during pharmaceutical product development. In: Tekade RK, editor. *Dosage Form Design Parameters*. Academic Press: London; pp. 1–30.

Maheshwari R, Tekade M, Sharma PA, Tekade RK. 2015. Nanocarriers assisted siRNA gene therapy for the management of cardiovascular disorders. *Current Pharmaceutical Design*. 21(30):4427–4440.

Mandal A, Chakrabarti S. 2017. *Impact of Ion Implantation on Quantum Dot Heterostructures and Devices*. Springer: Singapore.

Månsson A, Sundberg M, Balaz M, Bunk R, Nicholls IA, Omling P, Tågerud S, Montelius L. 2004. *In vitro* sliding of actin filaments labelled with single quantum dots. *Biochemical and Biophysical Research Communications*. 314(2):529–534.

Matea CT, Mocan T, Tabaran F, Pop T, Mosteanu O, Puia C, Iancu C, Mocan L. 2017. Quantum dots in imaging, drug delivery and sensor applications. *International Journal of Nanomedicine*. 12:5421.

Medina DX, Householder KT, Ceton R, Kovalik T, Heffernan JM, Shankar RV, Bowser RP, Wechsler-Reya RJ, Sirianni RW. 2017. Optical barcoding of PLGA for multispectral analysis of nanoparticle fate *in vivo*. *Journal of Controlled Release*. 253:172–182.

Modi S, Higgs NF, Sheehan D, Griffin LD, Kittler JT. 2018. Quantum dot conjugated nanobodies for multiplex imaging of protein dynamics at synapses. *Nanoscale*. 10(21):10241–10249.

Moondra S, Maheshwari R, Taneja N, Tekade M, Tekadle RK. 2018. Chapter 6 – Bulk level properties and its role in formulation development and processing. In: Tekade RK, editor. *Dosage Form Design Parameters*. Academic Press: London; pp. 221–256.

Morales-Narváez E, Montón H, Fomicheva A, Merkoçi A. 2012. Signal enhancement in antibody microarrays using quantum dots nanocrystals: application to potential Alzheimer's disease biomarker screening. *Analytical Chemistry*. 84(15):6821–6827.

Murphy-Royal C, Dupuis JP, Varela JA, Panatier A, Pinson B, Baufreton J, Groc L, Oliet SH. 2015. Surface diffusion of astrocytic glutamate transporters shapes synaptic transmission. *Nature neuroscience*. 18(2):219–226.

Nifontova G, Ramos-Gomes F, Baryshnikova M, Alves F, Nabiev I, Sukhanova A. 2019. Cancer cell targeting with functionalized quantum dot-encoded polyelectrolyte microcapsules. *Frontiers in Chemistry*. 7:34.

Olerile LD, Liu Y, Zhang B, Wang T, Mu S, Zhang J, Selotlegeng L, Zhang N. 2017. Near-infrared mediated quantum dots and paclitaxel co-loaded nanostructured lipid carriers for cancer theragnostic. *Colloids and Surfaces B: Biointerfaces*. 150:121–130.

Olson J, Mandava N. 2014. Method for stimulating retinal response using photoactive devices. Google Patents.

Olson JL, Velez-Montoya R, Mandava N, Stoldt CR. 2012. Intravitreal silicon-based quantum dots as neuroprotective factors in a model of retinal photoreceptor degeneration. *Investigative Ophthalmology & Visual Science*. 53(9):5713–5721.

Omidi M, Hashemi M, Tayebi L. 2019. Microfluidic synthesis of PLGA/carbon quantum dot microspheres for vascular endothelial growth factor delivery. *RSC Advances*. 9(57):33246–33256.

Parak WJ, Pellegrino T, Plank C. 2005. Labelling of cells with quantum dots. *Nanotechnology*. 16(2):R9.

Paris-Robidas S, Brouard D, Emond V, Parent M, Calon F. 2016. Internalization of targeted quantum dots by brain capillary endothelial cells *in vivo*. *Journal of Cerebral Blood Flow & Metabolism*. 36(4):731–742.

Peng C-W, Li Y. 2010. Application of quantum dots-based biotechnology in cancer diagnosis: current status and future perspectives. *Journal of Nanomaterials*. 2010, Article ID 676839.

Rosenthal SJ, Chang JC, Kovtun O, McBride JR, Tomlinson ID. 2011. Biocompatible quantum dots for biological applications. *Chemistry & Biology*. 18(1):10–24.

Ryan SG, Butler MN, Adeyemi SS, Kalber T, Patrick PS, Thin MZ, Harrison IF, Stuckey DJ, Pule M, Lythgoe MF. 2019. imaging of X-ray-excited emissions from quantum dots and biological tissue in whole mouse. *Scientific Reports*. 9(1):1–10.

Samuel SP, Santos-Martinez MJ, Medina C, Jain N, Radomski MW, Prina-Mello A, Volkov Y. 2015. CdTe quantum dots induce activation of human platelets: implications for nanoparticle hemocompatibility. *International Journal of Nanomedicine*. 10:2723.

Sarkar N, Sahoo G, Das R, Prusty G, Swain SK. 2017. Carbon quantum dot tailored calcium alginate hydrogel for pH responsive controlled delivery of vancomycin. *European Journal of Pharmaceutical Sciences*. 109:359–371.

Sarwat S, Stapleton F, Willcox M, Roy M. 2019. Quantum dots in ophthalmology: a literature review. *Current Eye Research*. 44(10):1037–1046.

Sharma PA, Maheshwari R, Tekade M, Tekade RK. 2015. Nanomaterial based approaches for the diagnosis and therapy of cardiovascular diseases. *Current Pharmaceutical Design*. 21(30):4465–4478.

Shi X, He D, Tang G, Tang Q, Xiong R, Ouyang H, Yu C-y. 2018. Fabrication and characterization of a folic acid-bound 5-fluorouracil loaded quantum dot system for hepatocellular carcinoma targeted therapy. *RSC Advances*. 8(35):19868–19878.

Shi Y, Pramanik A, Tchounwou C, Pedraza F, Crouch RA, Chavva SR, Vangara A, Sinha SS, Jones S, Sardar D. 2015. Multifunctional biocompatible graphene oxide quantum dots decorated magnetic nanoplatform for efficient capture and two-photon imaging of rare tumor cells. *ACS Applied Materials & Interfaces*. 7(20):10935–10943.

Singh PA, Bajwa N. 2016. Nano technical trends for cancer treatment: quantum dots a smart drug delivery system. *International Journal of Pharmaceutical Sciences and Research*. 7(4):1360.

Sun X, Li W, Zhang X, Qi M, Zhang Z, Zhang X-E, Cui Z. 2016. *In vivo* targeting and imaging of atherosclerosis using multifunctional virus-like particles of simian virus 40. *Nano Letters*. 16(10):6164–6171.

Taylor RD, Heine M, Emptage NJ, Andreae LC. 2018. Neuronal receptors display cytoskeleton-independent directed motion on the plasma membrane. *Iscience*. 10:234–244.

Tekade RK, Maheshwari R, Soni N, Tekade M, Chougule MB. 2017. Chapter 1 – Nanotechnology for the development of nanomedicine A2 – Mishra, Vijay. In: Kesharwani P, Amin MCIM, Iyer A, editors. *Nanotechnology-Based Approaches for Targeting and Delivery of Drugs and Genes*. Academic Press: Amsterdam; pp. 3–61.

Thorne RG, Nicholson C. 2006. In vivo diffusion analysis with quantum dots and dextrans predicts the width of brain extracellular space. *Proceedings of the National Academy of Sciences*. 103(14):5567–5572.

Uehara T, Ishii D, Uemura T, Suzuki H, Kanei T, Takagi K, Takama M, Murakami M, Akizawa H, Arano Y. 2010. γ-glutamyl PAMAM dendrimer as versatile precursor for dendrimer-based targeting devices. *Bioconjugate Chemistry*. 21(1):175–181.

Valley CC, Arndt-Jovin DJ, Karedla N, Steinkamp MP, Chizhik AI, Hlavacek WS, Wilson BS, Lidke KA, Lidke DS. 2015. Enhanced dimerization drives ligand-independent activity of mutant epidermal growth factor receptor in lung cancer. *Molecular Biology of the Cell*. 26(22):4087–4099.

Varela JA, Dupuis JP, Etchepare L, Espana A, Cognet L, Groc L. 2016. Targeting neurotransmitter receptors with nanoparticles *in vivo* allows single-molecule tracking in acute brain slices. *Nature Communications*. 7(1):1–10.

Wadia JS, Dowdy SF. 2005. Transmembrane delivery of protein and peptide drugs by TAT-mediated transduction in the treatment of cancer. *Advanced Drug Delivery Reviews*. 57(4):579–596.

Wagner AM, Knipe JM, Orive G, Peppas NA. 2019. Quantum dots in biomedical applications. *Acta Biomaterialia*. 94:44–63.

Wansapura PT, Dassanayake RS, Hamood A, Tran P, Moussa H, Abidi N. 2017. Preparation of chitin-CdTe quantum dots films and antibacterial effect on *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Journal of Applied Polymer Science*. 134(22):44904.

Weng KC, Noble CO, Papahadjopoulos-Sternberg B, Chen FF, Drummond DC, Kirpotin DB, Wang D, Hom YK, Hann B, Park JW. 2008. Targeted tumor cell internalization

and imaging of multifunctional quantum dot-conjugated immunoliposomes *in vitro* and *in vivo*. *Nano Letters*. 8(9):2851–2857.

Xu G, Mahajan S, Roy I, Yong K-T. 2013. Theranostic quantum dots for crossing blood–brain barrier *in vitro* and providing therapy of HIV-associated encephalopathy. *Frontiers in Pharmacology*. 4:140.

Yaghini E, Turner HD, Le Marois AM, Suhling K, Naasani I, MacRobert AJ. 2016. *In vivo* biodistribution studies and *ex vivo* lymph node imaging using heavy metal-free quantum dots. *Biomaterials*. 104:182–191.

Yang HY, Fu Y, Jang M-S, Li Y, Lee JH, Chae H, Lee DS. 2016. Multifunctional polymer ligand interface CdZnSeS/ZnS quantum Dot/Cy3-labeled protein pairs as sensitive FRET sensors. *ACS Applied Materials & Interfaces*. 8(51):35021–35032.

Zayed DG, AbdElhamid AS, Freag MS, Elzoghby AO. 2019. Hybrid quantum dot-based theranostic nanomedicines for tumor-targeted drug delivery and cancer imaging. *Future Medicine*.

Zhang M, Bishop BP, Thompson NL, Hildahl K, Dang B, Mironchuk O, Chen N, Aoki R, Holmberg VC, Nance E. 2019. Quantum dot cellular uptake and toxicity in the developing brain: implications for use as imaging probes. *Nanoscale Advances*. 1(9):3424–3442.

Zhang P, Lu H, Chen J, Han H, Ma W. 2014. Simple and sensitive detection of HBsAg by using a quantum dots nanobeads based dot-blot immunoassay. *Theranostics*. 4(3):307.

Zhang X, Guo Q, Cui D. 2009. Recent advances in nanotechnology applied to biosensors. *Sensors*. 9(2):1033–1053.

Zhao M-X, Zeng E-Z. 2015. Application of functional quantum dot nanoparticles as fluorescence probes in cell labeling and tumor diagnostic imaging. *Nanoscale Research Letters*. 10(1):1–9.

Zhao Y, Shaffer TM, Das S, Pérez-Medina C, Mulder WJ, Grimm J. 2017. Near-infrared quantum dot and 89Zr dual-labeled nanoparticles for *in vivo* Cerenkov imaging. *Bioconjugate Chemistry*. 28(2):600–608.