Quantum dot: magic nanoparticle for imaging, detection and targeting

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Abstract. Quantum dots (QDs) are one of the nanoparticles that use in Imaging, Detection and Targeting. Quantum dots are nanometer-size luminescent semiconductor crystals and have unique chemical and physical properties due to their size and their highly compact structure. They emit different wavelengths over a broad range of the light spectrum from visible to infrared, depending on their size and chemical composition. Eventual use of quantum dots to dramatically improve clinical diagnostic tests for the early detection of cancer. The use of quantum dots heralds a revolution in biological imaging. The current and widely used organic fluorophores have two shortcomings associated with their fluorescence. Signals from the labeled molecules can be obscured by cell autofluorescence, occurring in the visible spectrum and by photobleaching which seriously limits observation time. Colloidal quantum dots are bright, photostable fluorophores of a few nanometers in diameter. Because their size approximates that of individual biomolecules, water-soluble quantum dot complex have been used to target and image tumor cells. Despite their advantages the best materials for quantum dots; cadmium sulfide, CdS and cadmium selenide, CdSe can be highly toxic. While enhancing the biocompatibility of this nanoparticle various encapsulation techniques have also aided in their water-dispersibility and functionalization. QDs were introduced to cell biology as alternative fluorescent probes in recent years. Traditional fluorophores, e.g. organic dyes and fluorescent proteins are limited by their narrow absorption range, broad emission spectra and short fluorescent lifetime. (www.actabiomedica.it)

Key words: Quantum dot, nanoparticle, imaging, detection, targeting

Quantum dots

Quantum dots are spherical nano-sized crystals. They can be made of nearly every semiconductor metal (e.g., CdS, CdSe, CdTe, ZnS, PbS), but alloys and other metals (e.g. Au) can also be used (1, 2). The prototypical quantum dot is cadmium selenide (CdSe). Quantum dots range between 2 and 10 nm in diameter (10 to 50 atoms). Generally, quantum dots consist of a semiconductor core, over coated by a shell (e.g., ZnS) to improve optical properties, and a cap enabling improved solubility in aqueous buffers (Figure 1).

Synthesis

In the 1980s traditional lithography-based techniques (a combination of electron beam lithography and etching) were used to make quantum dots. However, these quantum dots are only in the nanometre scale in one dimension. The other two dimensions are limited by the resolution of the lithography. In the early 1990s, quantum dots were mainly prepared in aqueous solution with added stabilizing agents. This procedure yielded low-quality quantum dots with poor fluorescence efficiencies and large size variations.

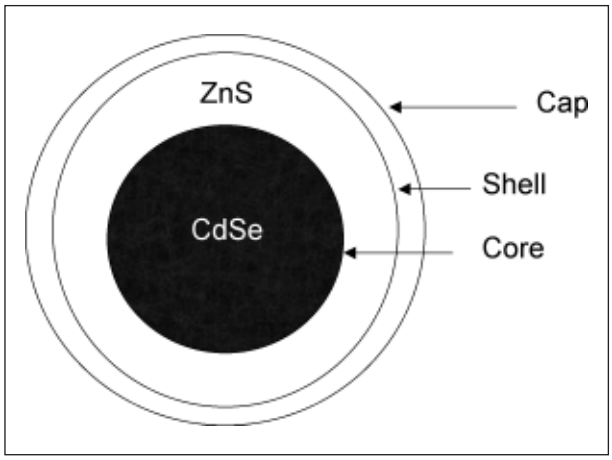

Figure 1. Schematic representation of a quantum dot. The cadmium selenide core is surrounded by a shell of zinc sulphide. Finally, a cap can be encapsulate the binary quantum dot by different material such as silica. The diameter of quantum dots ranges between 2-10 nm

From 1993 onwards, the high-temperature organometallic procedure was used for growing quantum dots (3). This procedure yields nearly perfect crystal structures and narrow size variations, but the fluorescence is still relatively low. The deposition of a surface-capping layer such as ZnS or CdS was found to dramatically increase the fluorescence properties of CdSe nanocrystals (4). The resulting quantum dots are highly hydrophobic and only soluble in nonpolar solvents. The art of quantum dot synthesis is evolving as alternative precursor materials, such as CdO, can be used to prepare high quality CdS, CdSe, and CdTe nanocrystals (5). In contrast to traditional binary quantum dots, and core/shell nanocrystals, the quantum dots synthesized show excellent quantum yields without an inorganic capping layer. The size of the quantum dot can be controlled by temperature (>300˚C) and period of time, ranging from minutes to hours depending on the desired particle size (6).

Properties

Quantum dots take advantage of the quantum confinement effect, giving these nanoparticles unique optical and electronic properties. A theoretical framework for these properties was already described in 1982 by two research teams in the former Soviet Union (7, 8). Fluorescence semiconductor quantum dots offer advantages in that they have a tunable absorption spectrum, which is very broad, extending from the ultraviolet to a cut-off wavelength in the visible spectrum. Emission is confined to a narrow band and can also be tuned. Absorption and emission characteristics are dictated by size for binary quantum dots or by composition/internal structure independently of size for alloyed semiconductor quantum dots, such as CdSeTe (2). When illuminated, smaller binary quantum dots emit shorter wavelength, such as blue, whereas larger dots emit longer wavelength, such as red (Figure 2). Moreover, quantum dots have brighter emission and good photostability (9).

Quantum dots are rendered water-soluble using several synthesis strategies, such as water soluble ligands (10), silanization (11), organic dendrons (12), cysteines (13), dihydrolipoic acid (14), encapsulation with block-copolymer micelles (15), with amphiphilic polymers (16), amphiphilic polymers conjugated with poly(ethylene glycol) (17), and surface coating with phytochelatin-related peptides. All these synthesis strategies have effectively solubilized CdSe or CdSe/ZnS quantum dots. In addition, quantum dots can be conjugated to biological molecules such as proteins, oligonucleids, small molecules, etc. which are used to direct binding of the quantum dots to areas of interest for biolabelling and biosensing (10, 11). Quantum dot bioconjugates are often used as simple replacements for analogous conventional dye conjugates when superior performance is required to achieve lower limits of detection, more quantitative results, more photo-stable samples, or higher levels of multiplexability. In combination, these spectral properties, unmatched by any known organic dye fluorophore, permit the systematic generation of probes that have different biochemical specificities and can be excited and detected simultaneously. A variety of colours of quantum dots are now available commercially from Quantum Dot Corporation (Hayward, California, USA) and Evident Technology (Troy, New York, USA). Recently, Evident Technology has announced the introduction of the first commercially available non-heavy metal quantum dots for life science research. These new quantum dots, called T2-MP EviTags™, feature a ternary core consisting of indium

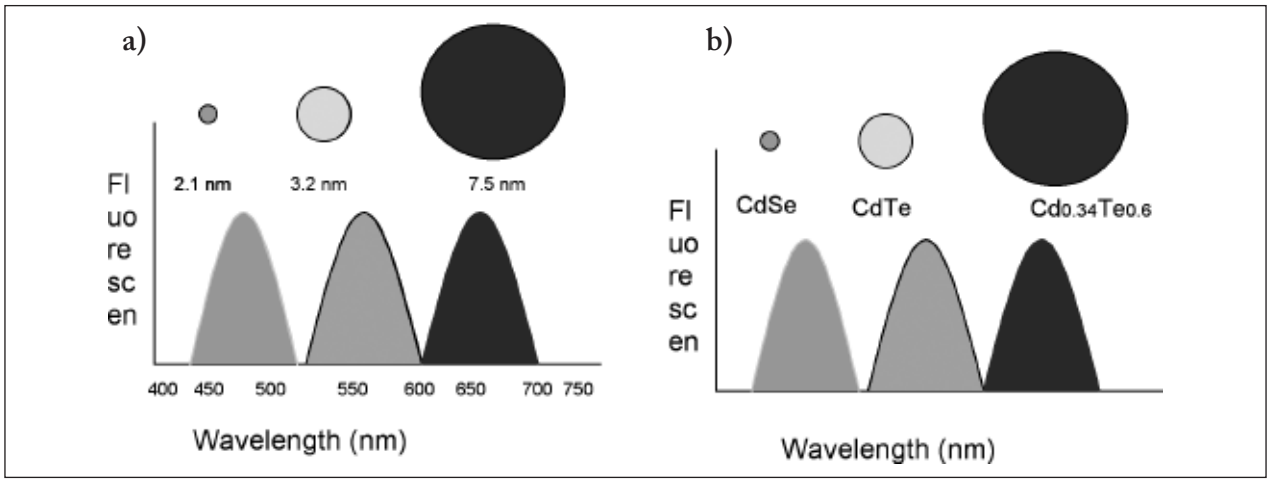

Figure 2. Optical properties of binary and alloyed quantum dots. Schematic drawings of three CdSe quantum dots with different diameters (a) and three quantum dots (mean diameter ~5 nm) with different composition (b) and their corresponding fluorescence emission spectra (2)

gallium phosphide coated with a metallic plating shell and a natural coating on the outer layer. The T2-MP EviTags™ offer a potential range of benefits over traditional quantum dots, especially the possibility of lower toxicity, and a wider range of colours into the near infrared.

Surface modifications

Since the QDs were coated with hydrophobic surfactant molecules, it can be only solved in organic solution. Before QDs can be applied to biological analysis, they have to meet several criteria. First, most of the biomolecules, e.g. protein, DNA, peptides exist in aqueous environment. Modifying the surface of QDs to be hydrophilic and compatible to varieties of biomolecules is important. Second, designing the techniques for specific labeling cells and biomolecules with QDs is necessary. Third, nontoxic performance of QDs is request for in vivo applications (14). Several strategies have been designed for hydrophilic bioconjugates of QDs One of the strategies employs functional group reactions. Primary amines, carboxylic acids, alcohols, and thiols are major reacting groups (10,11). Another method involves thiol-exchange reactions. Mercapto-coated QDs are mixed with Thiolated biomolecules, and chemical equilibrium is reached between absorbed thiols and the free thiols through overnight incubation (18, 19). On the other hand, some research group tagged QDs negative charged surfaces to an engineered recombinant protein with a polylysine chain via electrostatic interaction. Another different strategy employs phospholipid micelle to encapsulate QDs. The hydrophobic core of the micelle adsorbed to QDs through hydrophobic interactions. The hydrophilic terminals on the outer side of the micelle will interact with biomolecules (15). In addition, QD-tagged microbeads can be adapted to various biomolecules, providing optical codes for target molecules. All these strategies have been proved effective by in vitro or in vivo experiments (21, 22).

Quantum dots and Biomedical Research:

The applications of QDs in experiments reveal that QDs are sensitive, stable, nontoxic, versatile fluorescent probes. To target the peptide-labeled QDs to specific tissues and cell types in vivo is believed important for diagnostics and therapeutics (19). In the experiment, one group of QDs were labeled with peptides GFE, which recognize the membrane dipeptidase on the endothelial cells in lung blood vessels. Another group of QDs were labeled with pep-

tides F3, which bind to blood vessels and tumor cells in various tumors. The QD-GFEs were i.v. injected into normal mice. From the results, the QD-GFEs were only detected in the lung, but not in other tissues. QD-F3s were injected into the mice inoculated by tumors, QD-F3s were only observed in tumor blood vessels. No toxic effects were observed. Both experiments showed the specific targeting of cells and tissues in vivo by QDs. QD immunofluorescent labeling of different cellular targets (cell surface receptors, cytoskeleton components, and nuclear antigens) at different subcellular locations (surface, intracellular, and intranuclear) and with different types of specimens (cultured live cells, fixed cells, and tissue sections) was studied in recent years (16). In vivo cell tracing with QDs in early-stage Xenopus embryos revealed QDs were stable, nontoxic, and well resistant to photobleaching (15). The experiment also indicated QDs could be used to study cell differentiation and development in embryogenesis. Quantum-dots-tagged microbeads, a new tool for identifications of target biomolecules have been applied in multiplexed biological analysis (21, 22). Certain number of beads with precisely determined ratios of colors and emission intensities are inserted into a microbead. The microbead will give a specific optical code in fluorescent imaging. Based on the optical advantages of QDs, m kinds of colored QDs with n kinds of light intensities could compose n^m-1 microbeads with distinct optical codes. If probing biomolecules, the target biomolecules will be identified from the specific optical codes. So that is also called the "barcode" of the target molecule. This technique has been applied to gene expression analysis and more multiplexed bioanalysis. Although some aspects still need further investigation, fluorescent quantum dots have shown their great power in future biological analysis. Their applications in disease diagnostics, proteomics, genomics, drug delivery, and even drug candidates screening are foreseeable (18, 23). In recent years quantum dots have been increasingly tested for imaging of live cells. Numerous groups have demonstrated the practicality of quantum dots for both in vitro and in vivo studies. For example, the increased brightness and longevity of quantum dot fluorescence has been amply illustrated during live animal targeting and imaging. Long term imaging correlates well with the increasing molecular weight of polyethylene glycol (PEG) used for coating of the quantum dots (24). The high molecular weight PEG was shown to inhibit deposition of quantum dots in the liver, skin and bone marrow (24). Further, reducing the absorption by the reticuloendothelial system and thus increasing bloodstream circulation lifetime will be necessary for in vivo studies (24). Another group also exploited the use of polyethylene glycol as a block copolymer with phosphatidylethanolamine and phosphatidylcholine forming micelles in which single quantum dots could be encapsulated (13). These quantum dots enclosed in PEG-conjugated phospholipid micelles were injected into tadpole embryos and after 4 days showed no signs of toxicity, did not appear to aggregate and had much better resistance to photobleaching than other fluorophores, e.g. Rhodamine green-dextran (RG-D) (13). The targeting of blood vessels and cancer cells was first completed using quantum dots wherein the surfaces were conjugated with specific peptides, (19). To inhibit uptake by the reticuloendothelial system the quantum dot surface was co-coupled with polyethylene glycol (PEG) of molecular weight 5000. The peptide-coated quantum dots were shown to reach their vascular targets: both blood vessels and tumor cells and did not accumulate in surrounding tissue (19). It has been postulated that the quantum dot size with the peptide coating was sufficiently large to impede tissue penetration (19). The infiltration of surrounding tissue by organic fluorophores often obscures the intended target cells and results in images of inferior quality. Another study where quantum dots were encapsulated with an ABC triblock copolymer to which was linked a targeting ligand for tumor recognition reported the successful binding to human prostate cancer cells (25). The high molecular weight, triblock copolymer was meant to provide a stable, hydrophobic protective layer around the TOPO-capped quantum dot. In addition to the tumor targeting ligands and similar to earlier examples the polymeric surface was conjugated to polyethylene glycol molecules for improved biocompatibility and circulation. The optical properties of the quantum dots, e.g. absorption and emission spectra and fluorescence quantum yield were unaffected by the

extensive surface modification (25). These examples serve to illustrate that: 1) quantum dots appropriately modified with polyethylene glycol of sufficient molecular weight have sufficient stability to remain in circulation for days and in some cases months, 2) in parallel studies emission from quantum dots surpasses that of organic fluorophores in both brightness and duration, 3) conjugation of peptides to the quantum dot surface can be designed for targeting and imaging specific cancer cells and 4) coating strategies either in use or in development are likely to mitigate the toxicity of CdSe quantum dots in vivo (26).

Future and Important Objective

It is very important that develop a biocompatible quantum dot as a potential probe for many disease such as: neurological disorders. The starting material is a commercially available CdSe-ZnS core-shell quantum dot having surface ligands of tri-n-octyl phosphine oxide (TOPO). The quantum dots will be solubilized following the exchange of a thiol-containing molecule for the TOPO ligands. One such strategy involves the replacement of TOPO with either mercaptosulfonic acid or D, L cysteine (13). subsequently, a layer of the conductive polymer, polypyrrole will be attached through an electrostatic interaction. At this stage scientists have the option to also incorporate polyethylene glycol for enhanced circulation time should the quantum dots be tested later in vivo. The polymer polypyrrole will be the key to studies of the electrochemical stimulus that occurs between neurotransmitters and their corresponding receptors. The amine functionality of polypyrrole provides a favourable site for further addition reactions. Polypyrrole was chosen because of its electrically conducting properties, ease of preparation and its ability to conjugate with many amino acids. Most neurotransmitters derive from amino acids (as related compounds such as choline). Some neurons modify amino acids to form the "amine" transmitters, e.g. norepinephrine, serotonin and acetylcholine others combine amino acids to form "peptide" transmitters while still others neurons use amino acids unchanged or synthesized as transmitters (27). Preliminary investigations will begin with the binding of the amino acid tyrosine to the polypyrrole-coated quantum dots. Tyrosine is the precursor to the neurotransmitters dopamine (DA) and norepinephrine (NE). Subsequent studies will involve the conjugation of dopamine to polypyrrole-coated quantum dots. The loss of neurons and the accompanying decrease in levels of dopamine are associated with Parkinson's disease (27). Also dopamine is a good candidate as it is electroactive (28). Active laboratory preparation of materials will start with the solubilization of the quantum dots whereby a thiol-containing molecule is to be exchanged for TOPO. A measure of the completeness for the occurrence of this exchange will be determined by mass spectrometry (MALDI-TOF) and infrared spectroscopy. Absorption and emission spectra will be recorded throughout to monitor for any changes during the stepwise, surface modification of the quantum dots. Morphology and size determination will be completed using transmission electron microscopy and dynamic light scattering (DLS). Light scattering is the best estimator of the actual particle size as it measures the hydrodynamic radius. As an indication of potential toxicity, free Cd2+ will be measured by inductively coupled plasma (ICP) according to the methods of Derfus et al. (29). Whereas the binding of appropriately modified quantum dots to specific target cells can be followed and observed by fluorescence microscopy the interaction of chemicals comprising a neurotransmitter and receptor may prove difficult to confirm. Should this attachment between neurotransmitter and receptor not be brief it may be possible to verify using 1H NMR. Further, electrochemical stimulus to drive the interaction between neurotransmitter and receptor will be investigated using individual electrolytic solutions in which there is an excess of Na+, K+ and Ca+ ions. These ions are involved in the process of neurotransmission. This research project is expected to run for 2 to 3 years after which time a decision will be made on future direction. The materials synthesis and characterization will require two full-time students with interests in synthesis, biochemistry and analytical techniques. This project represents an opportunity for exposure to the exciting new field of bio-imaging with quantum dots.

Quantum dots-based optical imaging

Fixed cells and tissue imaging

The feasibility of using quantum dots for antigen detection in fixed cellular monolayers was first demonstrated in 1998 (11). By labelling nuclear antigens with green silica-coated CdSe/ZnS quantum dots and F-actin filaments with red quantum dots in fixed mouse fibroblasts, these two spatially distinct intracellular antigens were simultaneously detected. For cellular labelling quantum dots are ~20 times brighter and dramatically morephotostable over many weeks after injection than organic fluorophores (10). Recently, specific genomic sequences and antigens in tissue sections have been labeled (13).

Live cell bio imaging

Live cell imaging is a more difficult task compared to fixed cells and tissues due to the care that must be taken to keep cells alive and due to the challenge of delivering probes across the plasma membrane for studying intracellular targets. In vivo applications of quantum dots have been demonstrated for labelling cellular surface antigens (10). By covalently conjugating mercaptoacetic acid-coated CdSe/ZnS quantum dots to the transferrin protein, quantum dots were spontaneously endocytosed by cancer cells and retained their bright fluorescence, indicating that quantum dots can be used as intracellular labels. Forintracellular staining of cells poly(ethylene glycol)-coated CdSe/ZnS quantum dots with green emission were injected into single cells of a *Xenopus laevis* embryo (15). Microscopic fluorescence imaging allowed real-time monitoring of cell lineage and differentiation. Remarkably, most of the embryos exhibited normal development, and there was no evidence of toxicity, even with the injection of over one thousand million quantum dot particles per cell. Recently, the true advantages of quantum dots for live cell imaging have been demonstrated by labelling plasma membrane receptors, such as glycine receptors (30) and erbB/HER receptors (31) enabling real-time tracking of biomarkers and imaging single molecules. The data provide new insights into the mechanism of ligandreceptor interactions. Targeting of quantum dots to specific cytoplasmic or nuclear locations for monitoring biological events is a more difficult task as the plasma membrane barrier and the entrapment of quantum dots in the endocytic pathway has to be circumvented. Different mechanisms have been used to deliver quantum dots into the cells, such as microinjection (15), non-specific uptake of quantum dots through endocytosis (14), conjugation of quantum dots to translocating proteins (10) or cationic peptides, or specific membrane receptors (31). All these techniques have successfully delivered quantum dots into cells, although it seems that the peptide-mechanism may be the most efficient.

In vivo imaging

In order to benefit from the advantageous optical properties of quantum dots as *in vivo* labels, a number of issues must be addressed. First, the relatively large size and surface area of quantum dots allow the attachment of multiple targeting probes to each label of enhanced binding specificity. However, this size (~4-20 nm in diameter following bioconjugation) has the disadvantage of being too large to penetrate through the vascular endothelium, and too large to be excreted in the urine. The accessible targets for systemically administrated quantum dot probes could be limited to those of vascular exposure, such as endothelial receptors. Also, nanoparticles are non-specifically taken up by phagocytic cells in the organs of the reticulo-endothelial system (most notably by the liver and spleen). This non-specific targeting can be reduced by coating nanoparticles with hydrophilic polymers such as poly(ethylene glycol) to allow greater vascular circulation time, but non-specific uptake cannot be eliminated completely (17, 19). Quantum dots were first used to target tissue-specific vascular markers by intravenous injection in live mice (19). CdSe/ZnS quantum dots with either green or red emission were conjugated with tissue-specific peptides targeting lung blood vessels, tumour blood vessels, or tumour lymphatic vessels. Fluorescence visualization of mouse tissue showed uptake in the target tissue, but non-specific uptake by the reticulo-endothelial system was also observed (32). As long as

the target organ is part of the reticulo-endothelial system, such as a lymph node, this is not a problem. Targeting via transdermal injection has been shown in porcine sentinel lymph nodes using a near-infrared fluorescence imaging system (33). In this study near-infrared CdTe/CdSe quantum dots were used. Near-infrared light has the advantage to be attenuated less by biological tissue and manytypes of quantum dots have recently been developed with emission within this range (2). The injected quantum dots were phagocytosed non-specifically by dendritic cells which migrated to sentinel lymph nodes. The quantum dots could be followed visually to the lymph system even 1 cm under the skin surface of the animals. This new imaging technique allows surgeons to see clearly the target lymph nodes without cutting the animals' skin and is a significant improvement over the dye/radioactivity method currently used for several reasons. Throughout the procedure, the quantum dots are clearly visible allowing the surgeon to see not only the lymph nodes, but also the underlying anatomy (34, 35). The pathologist/surgeon can focus on specific parts that would be most likely to contain malignant cells, if cancer were present. The imaging technique minimized inaccuracies and permitted real-time confirmation of the total removal of the target lymph nodes, drastically reducing the potential for repeated procedures and unwanted trauma. The technique has not been applied in humans yet. Before quantum dot clinical applications become possible, the biocompatibility of these nanoparticles must be thoroughly investigated So far, nearly all of the publications on the in vivo use of quantum dots have reported normal organism development and no detectable toxicity (17-19). However, long term stability has not been investigated, and it is unlikely that systemically administered quantum dots will be completely excreted from the body prior to degradation. Recently, the cytotoxicity of CdSe/ZnS quantum dots with various coatings has been determined in cultured liver cells (29). The results showed that surface coatings must be sufficiently stable to prevent oxidation of the quantum dot surface, which results in the release of toxic and carcinogenic cadmium ions. For stability *in vivo*, the amphiphilic polymer coating results in a robust layer.

In vivo tumour targeting and imaging

Targeted molecular imaging of tumours was first demonstrated in nude mice using quantum dots (32). Nude mice lack a thymus and a functional immune system. Therefore, a human xenograft of tumour cells will be accepted and grow in nude mice. This xenograft tumour model is therefore an excellent model to study in vivo targeting of therapeutics to human cancer cells. Subdermal tumours require only a shallow penetration depth for imaging. Moreover, the vasculature of most cancer tissue is highly disordered, causing exposed interstitial tissue, so that tumour antigens are in direct contact with blood. Nude mice with human prostate tumours were injected intravenously with poly(ethylene glycol)-conjugated quantum dots functionalised with anti-bodies against the prostate-specific membrane antigen. Quantum dot accumulation in the tumour was primarily due to antibody-antigen binding, but was also aided by the enhanced permeability and retention effect characteristic for tumor vasculature. The permeability and retention effect is due to the inherent vasculature permeability of the microenvironment of cancerous tissue, combined with the lack of lymphatic drainage (36). Due to the permeability and retention effect alone, it was found that nonconjugated poly(ethylene glycol) quantum dots accumulated in induced mouse tumours, demonstrating tumour contrast, but much less efficiently than actively targeted probes. Recently, an intraoperative highly sensitive technique for pulmonary sentinel lymph node mapping using near-infrared fluorescent quantum dots has been developed. The study showed the feasibility of the method for mapping pulmonary lymphatic drainage and guiding excision of the sentinel lymph node in a porcine model. In addition, the application of quantum dots in multiphoton intravital microscopy shows great versatility for studying tumour pathophysiology. Intravital microscopy is a powerful imaging technique that allows continuous non-invasive monitoring of molecular and cellular processes in intact living tissue with 1-10 μm resolution. Quantum dots can be customized to concurrently image and differentiate tumour vessels from both perivascular cells and matrix and to monitor the trafficking of bone marrow-de-

rived precursor cells to the tumour vasculature allowing to investigate the degree to which the vascular and perivascular structures are formed or remodelled in response to cell homing.

Gao et al. (37) created QD conjugates containing a special polymer coating (including a QD capping ligand) for in vivo protection, targeting ligands for tumour recognition, and several molecules (poly ethylene glycol) for improved biocompatibility and circulation (Figure 3). By attaching a targeting ligand, high-affinity binding of QD-antibody conjugates to tumour antigens occurs (Figure 4).

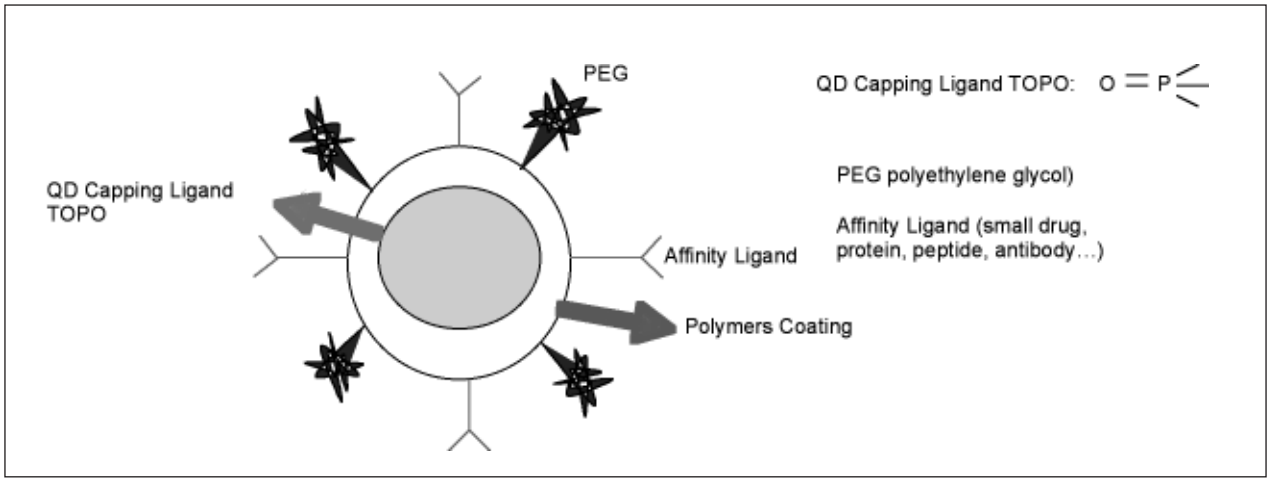

Figure 3. Structure of a Multifunctional Quantum Dot Probe*
* PEG, polyethylene glycol; QD, quantum dot; TOPO, tri-n-octylphosphine oxide

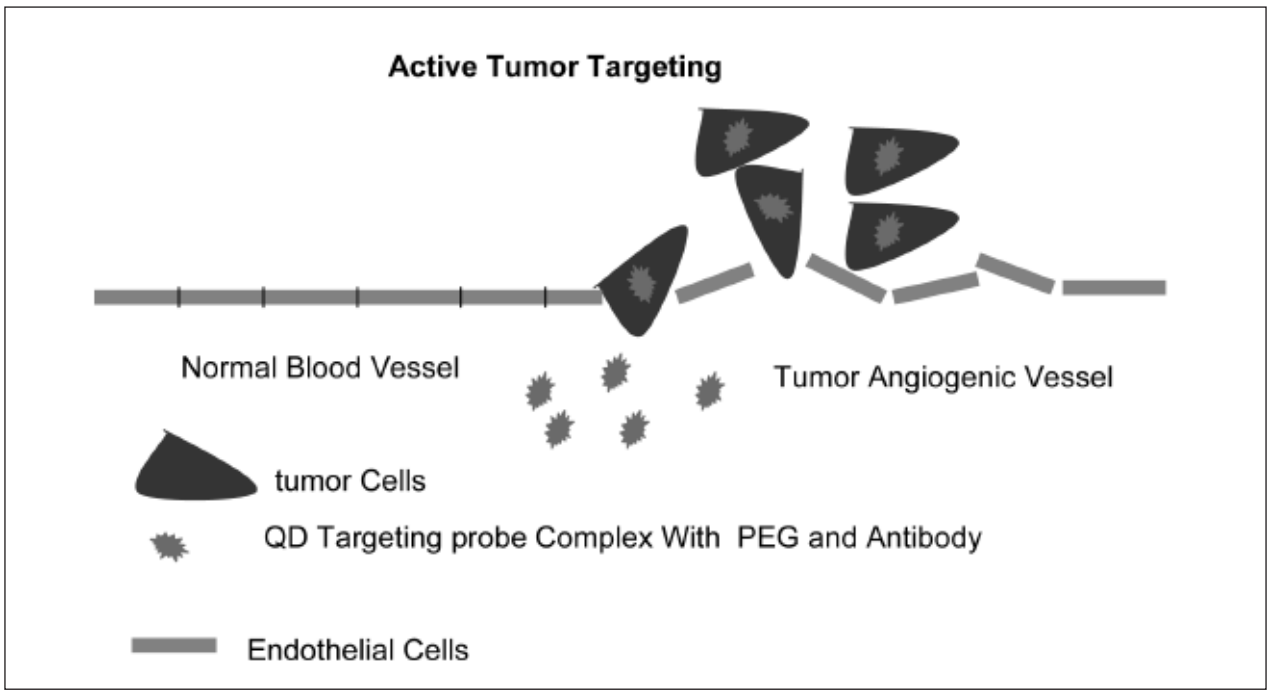

Figure 4. Structure of a Multifunctional Quantum Dot Probe*
* PEG, polyethylene glycol; QD, quantum dot; TOPO, tri-n-octylphosphine oxide

Conclusion

Quantum dots have been received as new technology with novel characteristics that could greatly developed biological imaging and detection. It can help to improve different field of biomedical sciences such as:

- Design and produce of nanoparticles and nanodevise with multiple functions
- Use of QD complex for analyzing of biomarkers and detection of disease.
- Design and make of biocompatible and biodegradable nanoparticles to sole the problem like nonspecific organ uptake and RES scavenging
- Deliver of nanoparticles for of imaging and therapeutic aim into solid tumors beyond the vascular endothelium

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