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Marvelous applications of quantum dots

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Abstract. – BACKGROUND: Nanotechnology is developed to convert research, prevention, and treatment of cancer through the novel diagnostic imaging techniques and therapies. In particular, the imaging nanotechnology has gained substantial momentum in recent years. The main progress in nanotechnology has led to the production of novel fluorescent probes named quantum dots (QDs). Quantum dots develop a revolution in the molecular tagging processes within research, *in vivo* and *in vitro* studies. Due to unique physical and chemical features of QD probes, new possible techniques of early cancer detection and therapeutic management are being surveyed. Quantum dots have also dissolved many of the restrictions of organic fluorophores and are a talented option as a research tool.

CONCLUSIONS: This review addresses on the present status of research, preclinical applications and also future visions of quantum dots.

Key Words:
Quantum dot (QD), Nanomedicine, Imaging, Treatment, Theranosctics.

Introduction

Semiconducting nanocrystal tools was primarily produced in the early 1980s in the labs Physical-Technical Institute in St. Petersburg[1,2]. The term "quantum dot" (QD) was first used by Mark A. Reed indicates nanocrystalline semiconducting fluorophores in 1988, whose excitons are detained in all three spatial dimensions and have typical diameters of 2-20 nm. Generally they are double systems created of a core of semiconducting material enclosed within a shell of another semiconductor. Quantum dots (QDs) fluorescence is produced by the bandgap between the valence and the conduction electron bands, and absorption of a photon higher in energy than the spectral bandgap of the core semiconductor results in electron excitation to the conduction band, generating an electron-hole pair (exciton). The long lifetime in the order of 10-40 ns augments the probability of absorption at shorter wavelengths, and develops a wide absorption spectrum. Because of the physical size of the bandgap ascertains the photon's emission wavelength, it is feasible to manage the fluorescence wavelength by the nanoparticle size (the bandgap energy is reversely relative to the square QD size). On other hand, the larger QD, causes redder emission. QDs have a number of exceptional physical features, mainly in the optical imaging. The unique properties such as size-tunable emission, narrow symmetric emission bands, strong light absorbance, bright fluorescence, high quantum yield, high photostability and low photobleaching rates, and their broad absorption spectrum cause increasing attention to preclinical and clinical applications. Chemical adjustment of the nanoparticle surface not only provides the particle water-soluble, lets biocompatibilization and functionalization as well as prevention of reactive oxygen formation. By adhering specific targeting molecules, QDs can be attached to subcellular structures or even organs in intact animals, so can be applied for bio-imaging goals in cells[3] and animals[4,5]. Also, QDs are currently being applied in bio-analytical assays[6] which are in research state. These strategies might be used in the diagnosis of the patients in future. Therapeutic applications such as photodynamic therapy[7], gene silencing[8,9], and drug delivery as well as simultaneous diagnosis and treatment (theragnosctics) are tested in lab-animals[10].

In vitro and in vivo Imaging with QDs

QDs have been successfully used in different imaging applications for *in vitro* and *in vivo* diagnostics due to these unique optical features[10]. The number of research investigations into QD biological applications after 1998 has increased exponentially[11,12]. QDs have been successfully applied as fluorescent tags for a different of bioanalytical aims, such as DNA detection, proteins, and other biomolecules[13-15], cellular labeling[11,12,16-21], and binding assays that use fluores-

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cence resonant energy transfer (FRET) to probe for target events[22-24]. Among the different applications of QDs, biomedical fluorescent imaging has attracted the greatest interest. The change in "color/size" of biofunctionalized QDs to target cells help to extend observation by fluorescent imaging with incessant illumination and multicolor imaging.

Biomedical research is classified in two *in vitro* and *in vivo* types. *In vitro* type renders to the handling of organs, tissues, cells, and biomolecules in a controlled and non-natural environment[25]. On other hand, *in vivo* studies refers to the depiction and survey on the biomolecules and biological systems in the intact organisms[25] and usually performs experiments in the large system of the animal. Here, we describe the recent fluorescent imaging investigation done *in vitro* and *in vivo*, which may help to implemented in the biomedical applications of QDs. *In vitro* fluorescent imaging mostly indicates to three categories: biomolecular tracking in cells, cellular imaging and tissue staining. In comparison with *in vitro* imaging, *in vivo* QD imaging encounters various challenges due to the increase in complexity resulting from multicellular organism and increasing in size. In total, there are four most important types of QDs *in vivo* imaging applications[26]: QDs biodistribution[27-31], vascular imaging[20,32-35], QDs tracking[21,36-49], and tumor imaging[18,50-59].

QDs and Multispectral Fluorescence Imaging (MSFI)

Multispectral imaging technologies have been extensively applied in the many fields such as astronomy, geology, agriculture, industry, and forensics[60,61] which mostly due to the enormous development of filters, detectors, data-analysis techniques, interdisciplinary approaches, and fluorescent dyes. Also, MSFI is a fast increasing field with applications in cell biology, preclinical drug development, and clinical pathology. Mixed MSFI with small-animal imaging and microscopy develops improved sensitivity, reliable quantization, and resolved multiple simultaneous signals[62,63]. MSFI is predominantly helpful for assessing objects that have several fluorescent labels that may have analogues RGB (Red, green, blue) color or that may be restricted in the similar or spatially overlapping compartments; this method is also efficient for evaluation objects that have physically powerful whole-animal autofluorescence[64-66]. QDs are unique nanocrystal fluorophores with steady wide excitation spectra, sharp and symmetric tunable emission spectra, enhanced brightness, superior photostability, and concurrent excitation of several fluorescence colors. MSFI-QDs can competently eliminate background and accurately demarcate weak spectral signatures to express highly sensitive and multiplexed imaging of molecular targets *in vivo*[67,68]. Full evaluations on MSFI-QDs in multiplexed immunohistochemistry and in situ hybridization (ISH) are accessible[69,70]. Another use of MSFI-QDs is as a reasonable alternate for microarray analysis. In this instance, multiplexed QD-labeled oligonucleotide probes can be applied for ISH in human sample biopsies. This system was examined in a the single and multiplexed QD-ISH samples of acute leukemia and follicular lymphoma. Spectral unmixing allows the dissociation of spatially colocalized signals. MSFI-QDs permit the quantitative categorization of multiple-gene expression, thereby making them helpful for the examination of human clinical tissues[71]. MSFI-QDs were also examined by streptavidin-conjugated QDs to sense up to 7 signals in tonsil and lymphoid tissues. Slides were examined using confocal laser scanning microscopy. Five streptavidin-conjugated QDs were applied on the same tissue part and could be analyzed concurrently on regularly processed sections (Figure 1). This multiplexing method has the potential to discover the clinically relevant multidimensional cellular communications that cause diseases[72].

QDs and Neuroscience

QDs represent a novel way with significant potential in neuroscience investigation. In addition to contribution a substitute to traditional immunocytochemistry, they are specifically precious for studies of neurons and glia. QDs can be applied to observe, compute, and track individual molecular occurrences using fluorescence microscopy, and they offer the capability to observe and track dynamic molecular processes over extensive times (e.g., from seconds to several minutes). For instance, QDs are valuable for showing of neuronal and glial interactions such as the tiny size of the synaptic cleft, or connecting an astrocyte process and a neuron. These features hardly attain by other techniques or methods. Due to their exceedingly small size and optical resolution, they are also most valuable for tracking the molecular dynamics of intracellular and/or intercellular molecular processes over long period scales. Nevertheless, it should be mentioned that the hydrodynamic radius of functionalized QDs is larger (15-20 nm) than their actual size of 5-8

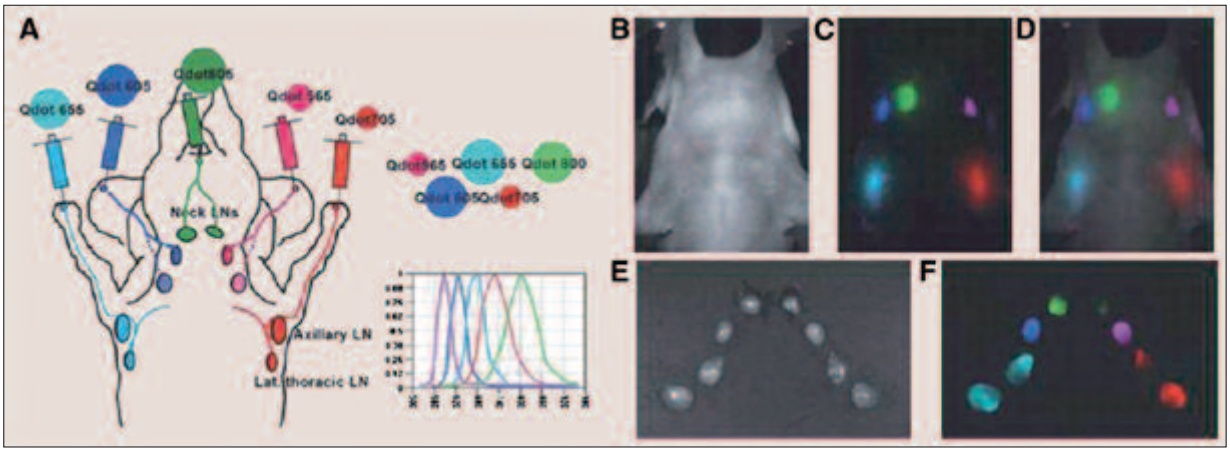

Figure 1. Five-color spectrally unmixed QD detection of lymphatic system anatomy. ***A,*** Schematic illustration of 5-color QD lymphatic injection sites and draining destinations of spectral fluorescence imaging. ***B,*** Autofluorescence image of mouse. ***C,*** Composite pseudocolored detection of draining lymph nodes after spectral unmixing. ***D,*** Image merge of *B* and *C*. Reflectance ***(E)*** and spectrally unmixed and pseudocolored ***(F)*** images of surgically dissected lymph nodes arranged in same geometry as in intact mouse. This figure originally appeared in an article by R.M. Levenson et al. in ILAR J 2008; 49: 78-88. It is reprinted with permission from the ILAR Journal, Institute for Laboratory Animal Research, The National Academies, Washington DC (www.national-academies.org/ilar).

nm[73]. Recent investigations of QDs in neuroscience demonstrated the potential applications of this technology. Triller at al[74] applied antibody functionalized QDs to follow the lateral diffusion of glycine receptors in cultures of primary spinal cord neurons. They could track the route of individual glycine receptors for tens of minutes at spatial resolutions of 5-10 nm, suggesting that the diffusion dynamics dependent on whether the receptors were synaptic, perisynaptic, or extrasynaptic. Vu et al[75] labeled nerve growth factor (βNGF) to QDs and applied them to improve neuronal-like discrimination in cultured pheochromocytoma 12 (PC12) cells. Lastly, these methods could be used to observe and follow functional responses in neurons. However, as same other new methods, there are limitations. For example, Vu et al[75] depicted that βNGF conjugated to QDs had decreased activity compared with free βNGF. Other groups are approaching the technology forward and developing new QD based techniques. Brinker et al[76] produced a technique to develop biocompatible water-soluble QD micelles that keep the optical properties of individual QDs. These micelles demonstrated uptake and intracellular dispersion in cultured hippocampal neurons. Ting et al[77] produced modified QD labeling methods that address the relatively large size of antibody-QD conjugates and the instability of some QD-ligand interactions. Their technique tags cell surface proteins with a specific peptide that can be straightforwardly biotinylated as a target for streptavidin-conjugated QDs. Using this approach, they were capable to in particular label and track AMPA (α-amino-3-hydroxy-5-methylisoxazale-4-propionate) receptors on cultured hippocampal neurons. Ultimately, QD nanotechnologies will need feasible approaches that can be straightforwardly copied in a typical neurobiology lab.

Photodynamic Therapy with QDs

Photodynamic therapy (PDT) is a novel management procedure for a various types of cancers[78]. In combination with surgical intervention, PDT has been applied productively in lung and gastrointestinal malignancies. This technique is previously a recognized treatment item in ophthalmology. During PDT, singlet oxygen is produced in the involved cells by a easy and controllable light-activated procedure. This photosensitizer course is capable of absorbing light of a suitable wavelength and utilizing that energy to stimulate oxygen to its singlet condition which induces apoptosis of cancer cells[79]. Selectivity is noteworthy in cancer treatment and has been utilized in PDT. Only cells which are concurrently in contact with the photosensitizer, light and in the attendance of oxygen are affected by the cytotoxic reactions[80]. In the work of Samia et al[81], CdSe QDs were connected to a silicon phthalocyanine (Pc4) photosensitizer through an alkyl group, and applied as a primary energy donor. Through the fluorescence resonance energy

transfer mechanism from QDs to the silicon Pc4 photosensitizer, reactive oxygen species (ROS) were produced for photodynamic cancer therapy. Furthermore, the semiconductor nanocrystals alone were established to generate ROS without a mediating photosensitizer.

QD: Unique Carrier for Drug Delivery

Accurate recognition of key molecular targets distinguishing not-healthy from healthy cells enables targeted drug delivery with negligible side-effects. Nanoparticle drug carriers demonstrate important features for efficient targeted delivery such as ufficiently long blood circulation, protection the cargo from degradation, large drug loading capacity, controlled drug release profile and integration multiple targeting ligands on their surface. Furthermore, QD probes afford novel functionality of traceable drug delivery, as biodistribution of carriers and intracellular uptake can be checked via fluorescence. Many drug delivery applications using QDs have been established recently. For instance, Chen et al[82] co-transfected QDs and siRNA using Lipofectamine 2000 and monitored transfection efficiency via QD fluorescence. Mixing QDs with transfection reagent in 1:1 mass ratio developed relationship between the QD signal intensity and the degree of gene silencing. Fascinatingly, more co-transfection of different siRNA molecules with various QD colors might develop multiplexed monitoring of gene silencing. More accurate quantitative data about the number of siRNA molecules delivered into cells can be attained by using QD-doped chitosan nanobeads demontarted by Tan et al[83]. In this method siRNA molecules are put downed on the surface of nanobeads, and intracellular delivery can be straightly monitored by the nanobead fluorescence. Further improvement can be gained using carbon nanotubes for intracellular delivery of antisense oligonucleotides tagged with QDs by Jia et al[84]. For example, straight tagging of plasmid DNA with QDs followed by Lipofectamine-mediated transfection enabled long-term survey of intracellular and intranuclear localization and transport of plasmid DNA, while preserving the ability of expressing reporter protein encoded by the plasmid[85]. Development of single-QD drug delivery vehicles for in vivo utilization is admirable, as intermediate size of such carriers (~10-20 nm in diameter) diminishes the renal clearance as well as uptake by reticulo-endothelial system (RES), thus rising the blood circulation time and improving the delivery efficiency. Further, QD core can provide as a structural scaffold for loading of various kinds of drug molecules (Figure 2)[86,87]. Flexibility of the shell plan enables engineering of drug carriers with various physical properties (e.g. size, charge, biodegradability, etc), thus, producing a large platform for a number of specific applications is allowed. *In vivo* drug delivery with QD carriers was established by Manabe et al[88]. Conjugation of an antihypertensive drug captopril to the QD surface developed the therapeutic effect same the free drug, while also enabling the monitoring of QD-drug biodistribution over a 96-hour period. With advancements in design of biocompatible QD surface coatings and identification of suitable molecular targets for therapy, QD-based drug delivery vehicles assure to present an crucial method for modeling of pharmacokinetics and pharmacodynamics of nanoparticle-drug carriers (Figure 2).

Future Perspectives

With the shared attempt of scientists in the fields of chemistry, biology, medical engineering and pharmaceutical sciences, QDs have been established as technological improvements with characteristics that could importantly progress *in vivo* and *in vitro* imaging and also gained important achievement in the research of nanomedicine. Nevertheless, a number of new questions have also been developed. However, in the near future, there are several areas that require to more attention[89]:

1. Production of QDs with higher biosafety. Although, the newly emerging carbon dots[90,91] carbogenic QDs[92,93] silica QDs[94,95] ZnO QDs[96,97] as novel types of safe and cheap luminescent QDs labels, have an promising prospect in the clinical applications.
2. The affect of labeling QDs on the inherent character of nanomedicines. For example, it is essential to differentiate of the pharmacokinetics and pharmacodynamics between QDs labeled and original drugs; the constancy and drug loading capacity variations of drug nanocarriers after QDs labeling.
3. Quantitative analysis for QDs fluorescence imaging. The current analysis is semiquantitative results, which cannot accomplish the quantitative prerequisite of biopharmaceutical analysis in living animals. This issue requires a multidisciplinary collaboration of pharmaceutical analysis, computer image processing and chemometrics[98].

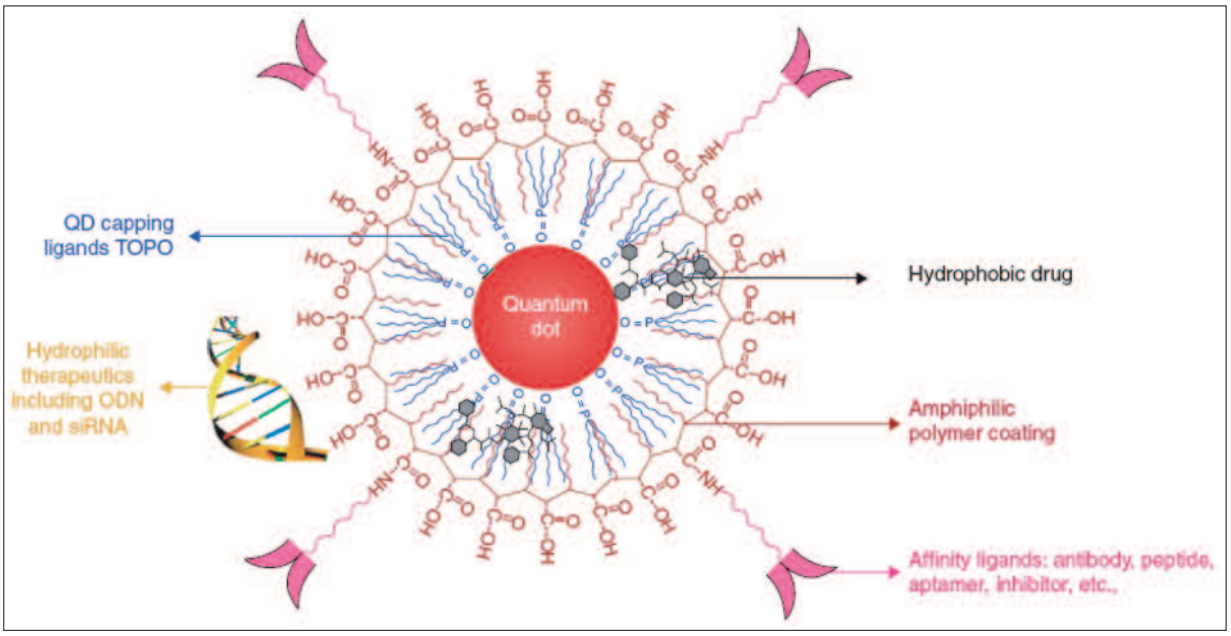

Figure 2. QD-based drug carriers integrate drug delivery tracing, loading of different types of drugs (e.g. hydrophobic small-molecule drugs between the QD core and polymer coating or hydrophilic drugs on the exterior surface of the polymeric shell), and targeting functionality. (Reproduced with permission from [Qi and Gao (2008)[87]].

Conflict of Interest

None declared.

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