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Standard Review

Emerging trends in nanobiotechnology

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Nanobiotechnology, an exciting interdisciplinary field of science, is making rapid progress in recent years with the development of new kinds of materials with all the desired physico-chemical properties needed for their successful application in various fields, in particular, medicine. Nanomaterials find applications in different thrust areas of medicine like therapeutics, diagnostics, surgical devices/implants, novel drug delivery systems etc. Recent advancements in this field include the development of semiconductor nanocrystals called "Quantum Dots" (QDs) and their very recent modifications called "Cornell Dots" (CU). Both QDs and CUs have extra-ordinary physico-chemical properties and have either low or no toxicity at all depending on the type of shell coated around the heavy metal. Of late, the toxic heavy metal core is also being replaced suitably for avoiding any potential risk during the long accumulation periods of these particles in biological tissues. This review focuses on the emerging trends in the development of wide array of nanomaterials for biological applications. The areas of emphasis include mainly the QDs - their properties, toxicity studies and some of their biological applications like labeling of cellular structures/molecules, cell uptake, biocompatibility, bioconjugation etc. Also, a short note is added on Cornell dots.

Key words: Nanobiotechnology, nanomaterials, quantum dots, Cornell dots, biological applications, biocompatibility, bioconjugation etc.

INTRODUCTION

"Nanobiotechnology", an extended term, can be defined as the Science and Engineering involved in the design, synthesis and characterization of non-toxic bioactive nanomaterials and devices which interact with cells and tissues at a molecular level with a high degree of specificity. These engineered materials and devices at the nanometer scale are constituted by molecules and atoms that were manipulated for specific and controlled physico-chemical properties.

The different synthetic methods of nanoengineered materials and devices, employing precursors from any of the three states of matter viz., solid, liquid and gas, have been broadly classified under "Top down" or "Bottom up" approaches. "Top down" technique encompasses the methodology of incorporating smaller-scale details into macroscopic material and is best exemplified by the photolithography technique used in the manufacture of integrated circuits by the semiconductor industry (Hu and Shaw, 1999).

The classic biological example of the lithographic technique is provided by the neuron-astrocyte communication studies (Takano et al., 2002). In this study, neuron and astrocyte cell cultures were placed in adjacent wells in agar that were connected by a channel made of poly dimethyl siloxane, allowing the diffusion of soluble factors. "Bottom-up" approaches, on the contrary, begin by designing and synthesizing custom-made molecules that have the inherent ability to self-assemble into structures of higher order. The critical part of this approach lies in the design of molecules which when subjected to physical/chemical trigger (change in pH, specific solute concentration, non-covalent interactive forces, application of electric field etc.) undergo self-assembly into macroscopic structures displaying desirable and unique physico-chemical properties that are not manifested by the constituents (Silva, 2004). The best examples for this

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approach come from the field of orthopedics. Bone is one type of connective tissue subjected to enormous use and abuse and consequent stress leading to frequent wear and tear. The repair process of this tissue is attempted in several studies using various types of artificial bone and biomaterials (Hartgerink et al., 2001; Stupp and Ciegler, 1992; Stupp et al., 1993). These materials are constantly being improved for better mechanical and cell signaling properties as well (Abbot and Cyranoski, 2003). Gradually nanomaterials are finding sustainable applications in almost every branch of medicine and diagnostics.

The major complications in building up nanomaterials come from their extremely minute size and time scales at the level of atomic bond oscillations. Therefore these studies warrant highly sophisticated theoretical and experimental tools. For visualization, characterization and manipulation of nanomaterials, novel physical characterization and sophisticated imaging techniques are under constant developpment. The ultimate aim of this technological development is to understand and evaluate the subtilities of intended interactions between the cells and the nanomaterials. Several significant studies are being carried out across the globe to harness the potential of the emerging field of nanotechnology for wide applications in Medicine such as development of novel drug delivery systems (Hanes et al., 1997; La Van Da et al., 2002; Lockman et al., 2002), porous self-assembling bilayer tubule systems (Schnur, 1993; Schnur et al., 1994), dendrimers that could be used as gene therapy agents or as contrast agents in diagnostic imaging (Karak and Maiti, 1997; Laus et al., 2003). Biological applications are innumerable ranging from the study of molecular motors such as flagella of bacteria (Imae and Atsumi, 1989; Noji et al., 1997) to the development of molecular computers (Adleman, 1994; Birge, 1995).

Visualization of structures and compartments within cells and molecules involved in biochemistry is not possible by routine microscopy as they are transparent to visible light and therefore molecules of interest have to be labeled with a marker. One of the common labeling techniques is Fluorescence labeling (Stephens and Allan, 2003; Weijer, 2003; Miyawaki et al., 2003) in which fluorophores can either be directly attached to target or can be attached to a molecule that binds to the target (Geiger and Volberg, 1994) by molecular recognition. Traditional fluorophores have limitations because of their conformational sensitivity to the local environment and consequent loss of fluorescence, inherent low resolution to optical microscopy as well as photobleaching (Pawley and Centonze, 1994). These limitations are to a considerable extent overcome by the use of colloidal metal particles particularly gold nanoparticles that give adequate contrast for imaging with electron microscopy. Yet again the disadvantage is that only fixed (dead) samples could be visualized. Therefore, in recent years, semiconductor nanocrystals, also called Quantum Dots (QDs) are introduced as a novel type of colloids for biolabeling, imaging and targeting. These QDs are nm size luminescent semiconductor crystals and have unique physical and chemical properties due to their size and 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. Since these are fluorescent, inorganic nanosolids, they can resist photobleaching and could be observed with high resolution by electron microscopy.

CHARACTERISTIC FEATURES OF NANOSYSTEMS

Nanosystems, mostly inorganic, are defined as nano-sized chemical objects with special features because of their quantum size and geometric effects. There are also inorganic-organic hybrid nanosystems and systems with specific applications in biology. Nanosystems, which are via media of isolated molecules with properties that follow quantum mechanical rules and the bulk materials that obey laws of classical mechanics, exhibit unique electronic, photochemical, electrochemical, optical, magnetic, mechanical or catalytic properties differing from those of molecular units and the macroscopic systems. The nanosystems are classified hierarchically as zero-, one-, two- and three dimensional nanosystems. Zero-dimensional systems include pseudo-spherical objects such as nanoclusters, nanoparticles or ceramic nanopowders. One dimensional systems account for carbon-based, metal-based or oxide-based systems in which the extension over one dimension is predominant over the other two. Ex: Solid nanowires, nanofibers or nanorods and hollow nanotubes. Two dimensional nanosystems are exemplified by the crystalline flat nanometric materials such as nanodiscs or nanoprisms and the amorphous nanofilms and nanomembranes. Finally, the three dimensional nanosystems, which can also be generated from simpler nanocomponents, consist of both crystalline and amorphous nanostructures such as nanocrystals and a wide array of ordered nanoarranged porous materials. The physical behaviour and optical responses of nanoparticles are determined by the wavelengths of light absorbed/scattered which in turn are dependent on the particle size and shape. Metallic nanoparticles (Au and Ag) are used as sensors to detect analytes through surface enhanced Raman scattering and other optical effects characteristic to the size range of 10 to 100 nm. There are several synthetic methods available in literature for the production of nanoparticles and these methods could be manipulated to control the particle size, morphology, crystallinity, shape and properties depending upon the intended application. Also, several kinds of materials like glass, metal etc. could be used for the production of nanoparticles.

Three dimensional super structures such as super lattices containing nanoparticles in predictable and periodic lattice points could be formed easily from 0-D

nanosystems by chemical interparticle interactions. The symmetry of the close-packed super lattice could be modulated by a careful control of the assembling parameters like particle size, shape and interparticle distances. Nanoporous materials, crystalline or non-crystalline display voids/pores ranging from 1 - 100 nm. Depending on pore size, they are categorized according to IUPAC as microporous (<2 nm), mesoporous (between 2 and 50 nm) and macromaterials (>50 nm). Inorganic-organic hybrid nanocomposites are a class of materials defined as inorganic nanostructures included in an organic matrix. Interestingly, the constituents of the hybrid system show special characteristics different from the ones they would have in the absence of the other. The organic matrices are mainly the polymers of varied compositions as they undergo phase transformation easily from fluid to solid and therefore could be moulded into desired shapes.

MAGNETIC NANOPARTICLES

These offer controlled size (a few nm to tens of nm) and are comparable to the dimensions of a cell (10 - 100 μm), a virus (20 - 450 nm), a protein (5 - 50 nm) or a gene (2 nm wide and 10 - 100 nm long). For this reason, the magnetic nanoparticles can be coated with biological molecules and make them interact with or bind to a biological entity. These particles could also be manipulated by an external magnetic field gradient. Since custom designed and fabricated multifunctional nanoparticles have wide applications in biology and medicine, researchers are currently modifying the magnetic nanoparticles by attaching them to antibodies, proteins, dyes etc. and / or integrate the magnetic nanoparticles by sequential growth / coating with quantum dots or metallic nanoparticles to attain multifunctionality like specific targeting of a drug to the region of tumour/cancer. The magnetic nanoparticles can also be made to heat up such that they could be used as hyperthermia agents, delivering toxic amounts of thermal energy to targeted bodies such as tumours or as chemotherapy and radiotherapy enhancement agents, where a moderate degree of tissue warming results in more effective malignant cell destruction. Conjugated magnetic nanoparticles with ligands, antibodies or proteins exhibit highly selective binding and show special properties like paramagnetism, fluorescence or enhanced optical contrast. Therefore these could be applied to biological/ medical problems such as protein purification, bacterial detection, toxin decorporation, enhanced medical imaging and controlled drug delivery. Heterodimer nanostructures with both magnetic and fluorescent properties would definitely serve as good candidates for dual-functional molecular imaging like a combination of MRI (Magnetic Resonance Imaging) and fluorescence imaging. Similarly, the integration of magnetic and metallic nanoparticles into heterodimers allows attachment of different kinds of functional molecules onto the specific parts of heterodimers, which then could bind to multiple receptors or act as agents for multimodality imaging. For example, yolk-shell nanostructures developed through encapsulation of a potential anti-cancer drug by iron oxide nanoshells serve as ideal nanodevices for controlled drug delivery.

Early detection of pathogens at their ultra low concentrations enables efficient clinical diagnosis and environmental monitoring. This is exemplified by the use of vancomycin-conjugated FePt nanoparticles to capture and detect pathogens such as vancomycin-resistant enterococci (VRE) and other gram +ve bacteria as well as Gram –ve bacteria at ultra low concentrations (Gu et al., 2003a, 2003b). It is reported that the detection limit achieved using FePt@Van magnetic nanoparticles matches with that of PCR (Polymerase Chain Reaction) based assays and in addition this protocol is faster and useful when PCR is inapplicable. Combination of FePt@Van biofunctional magnetic nanoparticles with fluorescence dyes provides quick, sensitive and low cost detection of bacteria in blood (Gao et al., 2006).

Protein purification is one critical step in down stream processing and greatly influences the economy of production of valuable biological products used in various industries, more particularly pharmaceutics. Research studies indicate that, of the existing protocols, magnetic separation and purification is a convenient method for selective and reliable capture of specific proteins, genetic materials, organelles and cells (Saiyeed et al., 2003; Safarik and Safarikova, 2004; Xu et al., 2004a, 2004b). Magnetic nanoparticles are also found to be of use in toxin decorporation as exemplified by the removal of UO_2^{2+} to the extent of 99% and 69% from water and blood respectively (Leroux, 2007). Conjugation of magnetic nanoparticles with organic dyes such as Cy5.5, FITC etc. will offer the multifunctional nanoprobes combining MRI and optical imaging (Cheon and Lee, 2008). A good example for this kind of bimodal imaging nanoprobe is obtained by the conjugation of Fe_3O_4 nanoparticles and porphyrin. Since porphyrin has low systemic toxicity and well understood pharmacokinetics, porphyrin derivatives are put to use in photodynamic therapy (PDT). Thus porphyrin modified Fe_3O_4 nanoparticles can act as a multifunctional nanomedicine that combine PDT anti-cancer treatment and non-invasive MRI imaging.

METALLIC NANOPARTICLES

Nanoparticles of metals like Au, Ag and Pt have excellent optical properties and hence could be used as optical contrast agents. They could also be used as multimodal sensors (a combination of optical and scattering imaging) and in photothermal therapy (Skrabalak et al., 2008).

Heterodimers of two distinct nanospheres like that of Fe_3O_4-Ag could be produced by sequential growth of metallic components (nucleation) on the exposed surface of the magnetic nanoparticles. Thus the general method for the formation of heterodimer nanoparticles is liquid-liquid interface heterogeneous growth (Gao et al., 2009). Another method to fabricate Fe_3O_4-Au heterodimers in a homogeneous organic solvent was reported in which $Fe(CO)_5$ is subjected to thermal decomposition onto the surface of Au nanoparticles and the following oxidation of intermediates produces uniform Fe_3O_4-Au heterodimers (Yu et al., 2005). This kind of multifunctional heterodimers (Fe_3O_4-Ag or Fe_3O_4-Au), along with the retention of their individual distinctive functionalities, can respond to magnetic forces, demonstrate enhanced resonance absorption and scattering and bind with specific receptors (Jiang et al., 2008; Xu et al., 2008).

YOLK-SHELL NANOSTRUCTURES

In conventional use of magnetic nanoparticles for drug delivery, they are coated with polymers and drugs are encapsulated to form nanocapsules or micelles (Gupta and Gupta, 2005). However, FePt@CoS_2 yolk-shell nanoparticles were developed as novel and potential nanodevices for controlled drug release in the treatment of cancer (Gao et al., 2007). Since FePt nanoparticles without any surface coating could act as potential anti-cancer drug like that of Cisplatin, the sequential growth of CoS_2 porous nanoshells by Kirkendall effect (Yin et al., 2004; Gao et al., 2006a) produced FePt@CoS_2 nanoparticles with an IC_{50} value lower than that of Cisplatin. Electron microscopic studies of these particles in vivo indicated that they are well taken up by cells and cellular organelles and that the FePt yolks disintegrate after the cellular uptake (Gao et al., 2009). Further developments include the production of FePt@Fe_2O_3 yolk-shell nanoparticles with dual functions viz., high cytotoxicity and strong MR contrast enhancement (Peng and Sun, 2007; Gao et al., 2008a). The essential characteristics in the above two kinds of nanoparticles that is FePt@CoS_2 and FePt@Fe_2O_3 for serving as effective cancer agents were their ability to release Platinum (II) species from the yolk and good permeability of shells to allow the metal ions to get released from the shells.

DENDRIMERS AND DIAMONDOIDS

Dendrimers are cylindrical structures providing new and unique properties that could be applied within the emerging field of nanotechnology (Zhang et al., 2003). These unique macromolecules generally possess multiple branches which can be used to carrya variety of agents. Dendronized polymers (with thickness in the range of several nanometers) composed of a linear polymeric backbone and dendritic side chains, attributed with high transfective efficiency and very low toxicity, could be used to form complexes with biomolecules that are to be delivered to cell and its constituents. Polyamidoamine (PAMAM) dendrimers have well defined surface functionality, good water solubility, low polydispersity and lack immunogenicity. For these properties, they serve as good candidates for use as the backbone of multitasking therapeutics (Choi et al., 2001). Similarly, Diamondoids are cage hydrocarbons with better therapeutic actions and less adverse effects. The smallest diamondoid molecule named adamantine and its derivatives can readily be synthesized and used for cancer treatment (Mansoori et al., 2007).

Liposomes

These are nanoscale closed vesicles consisting of a single lipid bilayer and are biodegradable. Liposomes are manufactured to encapsulate drugs for drug delivery like chemotherapy. The enclosed drug is delivered to targeted site only when the liposome adheres to the outer membrane of target cancer cells thus preventing drug toxicity to healthy cells (Silva et al., 2001; Torchilin and Weissig, 2003; Duncan et al., 2005).

QUANTUM DOTS (QD)

These are heterogeneous spherical nanocrystals constituted by a colloidal core and one or more surface coatings. They could be made of wide range of semiconductor metals like Cd, CdSe, CdTe, ZnS, PbS etc. and also by alloys and metals like Au. The size range of QDs is 2-10 nm in diameter (10 to 50 atoms). The surface coatings play a significant role in determining the QD applications as they prevent agglomeration, encapsulate toxic metals, affect stability in aqueous buffers, absorption and transport, modulate immunological responses, determine toxicity and aid in tissue elimination. QD could be customized for specific applications by varying their surface coatings (Ballou et al., 2004; Chang et al., 2006; Ghasemi et al., 2009). QDs are reported to have potential value in medicine, particularly in drug discovery due to their bright fluorescence, narrow emission, broad UV excitation, tunable size and high photostability (Tan et al., 2006). At the same time, they do pose challenges like biocompatibility, toxicity, photo-oxidation etc. which need to be addressed in detail for improving the applications of QDs in drug discovery.

Research on biomedical applications of QDs reveal that QDs are sensitive, stable, non-toxic, versatile fluorescent probes (Ghasemi et al., 2009). Specific targeting of biomolecule- labeled QDs *in vivo* (Ex: Peptide GFE labeled QDs recognize the membrane dipeptidase on the

endothelial cells in lung blood vessels; Peptide F3 labeled QDs bind to blood vessel and tumor cells) without toxic effects is achieved (Akerman et al., 2002). Another tool developed for the identification of target biomolecules is the QD-tagged microbead that gives a specific optical code for each molecule in fluorescent imaging (Han et al., 2001; Battersby et al., 2002). The microbeads are constituted by certain number of beads with predetermined ratios of colors and emission intensities. Suppose m kinds of colored dots with n kinds of light intensities are used, then $n^m - 1$ microbeads with specific optical codes could be composed. As the target biomolecules could be identified from the specific optical codes of microbeads, it is also called the "barcode" of the target molecule. This novel technique is applied to multiplexed bioanalysis including gene expression (Gao and Nie, 2001; Kralj and Pavelic, 2003).

Synthesis

Early synthesis of QDs employed traditional methods like a combination of electron beam lithography and etching limiting the particle size to the scale of nm only in one dimension but not in other two. Later QDs were prepared in aqueous solutions with added stabilizing agents but the quality of QDs so obtained was poor with low fluorescence efficiencies and large size variations. Then evolved the high temperature organometallic procedure for growing QDs with perfect crystal structures and narrow size variations. But as the fluorescence property did not improve deposition of surface-capping layer such as ZnS or CdS was employed that dramatically increased the fluorescence properties of CdSe nanocrystals. Alternative precursor materials such as CdO are also used to prepare high quality CdS, CdSe and CdTe nanocrystals. Further, the size of the QD could be controlled by high temperatures (>300^0C) and time duration ranging from minutes to hours depending on the desired particle size (Murray et al., 1993; Hines and Guyot-Sionnest, 1996; Peng and Peng, 2001).

Physico-chemical properties

Solubility in aqueous buffers

Synthesis of high quality nanocrystals of various semiconductor materials could be carried out in organic solvents at high temperatures in the presence of surfactants yields monodisperse and stable particles. This procedure produces nanoparticles with the polar surfactant head group attached to the inorganic surface and the hydrophobic chain protruding into the organic solvent mediating colloidal stability. The problem with these surfactant coated particles is that they are insoluble in water and hence limit their biological applications. This in turn could be overcome either by replacing the surfactant layer or by coating with an additional layer thereby introducing either electric charge or hydrophilic polymers for mediating solubility in water. In general, coulomb repulsion between nanocrystals of same surface charge prevents aggregation in water but in salt containing solutions such as cell culture media, charged particles tend to aggregate resulting in flocculation. On the other hand, use of hydrophilic polymers like PEG or dextrane involves steric stabilization such that vanderwaal forces leading to flocculation, as in the above case, does not happen here. In practice, hydrophobic nanocrystals can be made hydrophilic by exchanging surfactant coatings with ligand molecules which have reactive functional groups towards nanocrystal surface and hydrophilic groups on other end that ensure water stability. The anchoring groups used frequenty is thiol and carboxyl as hydrophilic head groups for pH 5. Mercaptohydrocarbonic acids like mercaptoacetic acid (Chan and Nie, 1998; Kloepfer et al., 2003), mercaptopropionic acid (Mitchell et al., 1999), synthetic peptides with multiple cysteines (Sukhanova et al., 2002; Sukhanova et al., 2004; Pinaud et al., 2004), Dithiothreitol (DTT) (Pathak et al., 2001), organic dendrons (Wang et al., 2002) and polyethylene glycol (PEG) (Skaff and Emrick, 2003) have been used for solubility in aqueous solution. In order to further stabilize the polymer shell around the nanocrystal, the individual polymer chains are cross-linked.

Photophysical properties

Energy difference between excited and ground state of a quantum dot strongly depends on its size. Upon optical excitation, electrons are excited from the valence shell to the conduction band which is analogous to exciting electrons from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO). The recombination of electron-hole pairs results in emission of fluorescence light. Typical water soluble nanocrystal comprises a semiconductor core, a shell of semiconductor material with a higher band gap and a hydrophilic coating to warrant water solubility. Organic fluorophores (Ex: Rhodamine, Fluorescein) can be optically excited within a narrow window of wavelengths and consequently the fluorescence emission is also limited to a certain window of wavelength. The fluorescence spectra of these organic fluorophores are not symmetric but exhibit a tail to longer wavelength called the "red tail". Contrastingly, colloidal quantum dots have a continuous broad absorption spectrum and the fluorescence can be excited with any wavelength shorter than the wavelength of fluorescence. Since the fluorescence emission spectra of QD are relatively narrow, symmetric and do not exhibit a red tail, many different colours can be distinguished without spectral overlap. This property is highly advantageous with respect to biological samples as their several different compartments /structures/processes could simultaneously be uniquely

labeled (Wu et al., 2003; Mattheakis et al., 2004). Thus several quantum dots with different colours of fluorescence can be excited with one single wavelength unlike the case with organic fluorophores. This flexible optical property of QD helps to reduce autofluorescence of biological samples by just selecting the appropriate excitation wavelength for which the autofluorescence is minimum. Further, the sensitivity of detection is greatly enhanced owing to the large separation between excitation and emission wavelengths leading to broad frequency windows in the emission spectra. The biggest advantage of QDs is their reduced tendency to photobleach because of their inorganic nature and this is useful for long term imaging such as fluorescence labeling of transport processes in cells or tracking the path of single membrane bound molecules (Dahan et al., 2003). Colloidal QDs are known to have long fluorescence time and interestingly, can also have fluorescence life times of a few tens of nanoseconds. If time-gated detection is employed that is, fluorescence is recorded after a few nanoseconds of optical excitation, then improved signal-to-noise ration could be achieved (Dahan et al., 2001). For this reason, CdSe/ZnS quantum dots have been used as contrast agents for imaging of blood vessels in living mice (Larson et al., 2003). Since colloidal QDs have a significant two photon cross-section, multiphoton excitation could be used for imaging of structures deep inside the biological systems. At this juncture, one should note that the quantum yield of hydrophilic QDs in aqueous solutions is lower than that of hydrophobic QDs.

Bioconjugation of QDs

Biomolecules can be fluorescence labeled by attaching quantum dots by one of the two strategies: First, biomolecules can be functionalized with a chemical group like mercapto group (-SH) that is reactive towards the semiconductor surface of materials like CdSe/CdS/CdTe /ZnS (Akerman et al., 2002; Rosenthal et al., 2002). The second strategy uses the covalent linking of biomolecules to the outer hydrophilic shell of the QD surface either nonspecifically or specifically by electrostatic interaction (Dubertret et al., 2002). Also, this could be done by means of cross-linker molecules which require the presence of hydrophilic surfactant shells with reactive groups such as –COOH, -NH_2 or –SH. Thus conjugation of QDs with biomolecules is well established for small molecules like biotin, folic acid, serotonin, avidin, albumin, transferring, trichosanthin, lectin, wheat germ agglutinin, antibodies and DNA. Streptavidin coated quantum dots are commercially avail-able and could be readily conjugated with biotinylated proteins and antibodies (Parak et al., 2005).

There are several alternatives in QDs with NIR emission for in vivo imaging compared with organic fluorophores. These special properties of QDs have inspired the fabrication of hybrid nanostructures that exhibit both fluorescence and magnetism such as Co@CdSe core-shell nanocomposites (Kim et al., 2005) and FePt-ZnS nanosponges (Gu et al., 2005). The combination of super paramagnetism and fluorescence at nm scale should help the biological applications of multifunctional nanomaterials. As the fluorescence of QDs is partially quenched by metallic nanoparticles in FePt-CdX hybrid nanostructures, their replacement with metal oxide nanoparticles results in a good quantum yield. It is shown that the Fe_3O_4 – CdSe heterodimer nanoparticles exhibit the emission wavelength peak at 610 nm with quantum yield of about 38% and these resulting fluorescent magnetic nanoparticles have both superparamagnetism and fluorescence of high quality. Hence the intracellular movements of these particles could be controlled using magnetic force and monitored using a fluorescent microscope (Gao et al., 2008). The magnetic nanoparticles drift to the magnet (external/applied magnetic force) due to the magnetic field gradient (H) and their significant magnetic moment. Although the movement of the magnetic nanoparticles inside the cell is slowed down due to high viscosity of cytosol, yet when the particles approach each other, the magnetic dipolar-dipolar (F_D – $_D$) interactions become the dominant forces and cause aggregation of nanoparticles inside the cells.

Efficacy of conjugated biomolecules with QDs should be experimentally verified for their potential applications in biology since it has been shown that the hybridizing ability of the oligonucleotides, bound to gold (Au) nanoparticles, with their complementary molecules is reduced (Demers et al., 2000). One of the major applications of QD in cell biology is their use as fluorescent markers for labeling of cellular constituents. This is achieved by conjugating receptor-specific ligand molecules such as antibodies to QD for cell surface target recognition and binding. It means that the conjugation should not hamper the molecular recognition mechanism. Various experimental studies using QDs demonstrated that the molecular recognizing abilities of the bound ligands are not compromised (Parak et al., 2005). Studies on neurotransmission, ion transport, enzyme catalysis etc. revealed that the functionality of biomolecules on conjugation with QDs is not significantly reduced and hence could be of vital importance in biological studies (Zhang et al., 2000, Kloepfer et al., 2003). However, contradictory results have also been reported by some investigators as in the case of serotonin-QD conjugate showing drastic reduction in its binding affinity to serotonin-transporter proteins (Rosenthal et al., 2002). Further research studies could alone reveal the extent to which the QD-bioconjugates could be useful in biological applications.

Biocompatibility of QDs

Colloidal QDs constituted by elements like lead, selenium,

cadmium etc. become toxic when they lose their hydrophilic shell coating. However, some cells have developed mechanisms to assemble these toxic ions as a part of biomineralizaton process. Toxicity studies on colloidal QDs which include the study of response of biological material to the presence of toxic ions and the stability of hydrophilic shell coating on the surface of QDs are vital for their enhanced bioapplications. A few investigations have already been carried out on the early embryonic stages of *Xenopus* and the results suggest that the dosage of the introduced particles / cell decide the extent of cellular and phenotypic abnormalities like variations in cell size, movement, axis elongation etc. There are also good number of reports suggesting no harmful effect of colloidal QDs tested on cells grown at various concentrations in culture media which is an encouraging sign for developing more applications for the colloidal QDs in cell proliferation and adhesion studies (Jaiswal et al., 2003; Winter et al., 2001). Colorimetric assays such as MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) were used by investigators to measure the survival rate of cells on incubation with coated toxin (CdSe/ZnS) containing QDs (Mattheakis et al., 2004). Similar other studies revealed no interference of QDs with cellular functions like ligand binding to cellular compartments, protein trafficking, signal transduction etc. It is quite evident from the results of various experiments that the harmful effects on cells are directly proportional to the extent of ion (Cd^{2+}) release (Aldama et al., 2001; Rikans and Yamano, 2000) and therefore appropriate stable encapsulation (like adsorption with bovine serum albumin, BSA) of the QDs would definitely suppress their cytotoxic effects (Derfus et al., 2004). Yet it is imperative that materials other than Cd, Se etc. should be explored for better utilization as no encapsulation mechanism would confer zero degradation on the particles which tend to remain for several years in biological tissues.

Labeling of cellular structures

Fluorescence microscopy is a widely used technique for visualization of molecules and cellular structures (Bruchez et al., 1998) present at the surface (Dahan et al., 2003; Lidke et al., 2004), and for labeling of intracellular constituents, the fluorescence labeled ligands will have to be artificially introduced by any one of the different methods like microinjection, electroporation etc. Researchers interested in these studies could use any of the standardized protocols available in literature (Herzog et al., 1994; Osborn, 1994). If antibodies are used as ligands, first a primary antibody is reacted with the target and then a biotinylated secondary antibody labeled by streptavidin-quantum dot conjugate via biotin-avidin interaction is an advantageous methodology since biotinylated antibodies are commercially available and a universal conjugation of QDs with streptavidin is adequate enough. For signal amplification, TSA enzyme amplification technique in which the secondary antibody is conjugated with horse-radish peroxidase and quantum dots conjugated to tyramide could be employed (Ness et al., 2003). Although colloidal quantum dots have an edge over organic fluorophores, yet the former cannot completely replace the latter.

Uptake of quantum dots by living cells

After the first report by the Chan and Nie group (1998) on the uptake and incorporation of QDs by living cells, several investigators made similar observations. The detailed study of this property of living cells throws light on the cellular communication modes for functions like exo and endocytoses apart from other functions like the processing of nutrients, uptake of viruses, drugs, nucleic acids and gene delivery. It is not enough if one can make the cells ingest QDs and fluoresce but they should be guided through the membrane into the nucleus for interaction with the chemical molecules like DNA, mRNA etc. as in the case of drug/gene delivery. Release of these QDs from internalized vesicles could be mediated through various methods like use of membrane-disrupting peptides (Wagner et al., 1992; Plank et al., 1994, 1998) and osmotic destabilization (Sonawane et al., 2003). With the use of fluorescein labeled dextrane as marker, Hanaki et al. (2003) showed that endosomes/lysosomes colocalize with ingested quantum dots. Further Jaiswal et al. (2003) demonstrated that quantum dot uptake could be blocked by cooling the cells to 4°C. Interestingly, the ingested QDs were found to be distributed between daughter cells during cell division suggesting an analogy to the nucleic acid delivery. This property could be made use of for arresting the cellular proliferation in malignancies through appropriate drug coated QD delivery.

Cell tracking

QDs by virtue of their optical properties can highlight cells and subcellular structures and hence can be classified as “Labels” or “Contrast agents” for they can greatly enhance the signal-to-noise ratio in medical diagnostic imaging procedures. When used for this purpose, QDs should possess the properties of molecular recognition facilitating the display of miniature structures and/or physiological processes and meet the safety requirements laid down for any labeling substance. “Cell tracking” is an important area of study in developmental biology wherein the cellular predetermination, differentiation, de-differentiation/re-differentiation, cell lineage (fate maps) and “Homing” of embryonic cells are studied at cellular and molecular level. Also, cell tracking is essential for understanding the molecular mechanisms

underlying the development of dreaded cellular malfunctions such as cancer development and metastasis. In all these studies, “cell labeling” is done with microinjection of oil drops/organic fluorophores or transfecting cells with genes for fluorescent proteins like GFP (Tombolini and Jansson, 1998). In recent years, QDs are being tested for their suitability and efficacy as markers (Dubertret et al., 2002) in embryonic development.

“Cell migration” (morphogenetic movements) and “Cell adhesion” are phenomena that play essential role in designing biocompatible surfaces of medical implants which are relevant in orthopedics and in tissue engineering. First studies on cell migration came to light through a popular method called Albrecht-Buehler “Phagokinetic track” method to follow cellular migration on cell culture substrates (Albrecht-Buehler, 1977a; Albrecht-Buehler, 1977b; Albrecht-Buehler and Lancaster, 1976). Originally Au particles were used as markers and were visualized by dark field microscopy (Albrecht-Buehler and Lancaster, 1976) and by Transmission Electron Microscopy (TEM). Later colloidal gold polystyrene microbeads were employed (Obeso and Auerbach, 1984) and presently QDs are put to fruitful use for recording phagokinetic tracks. The advantages of QDs are that they allow tracking the migration behaviour of cells in three dimensional cultures as they could be observed in stacked layers with different colours of fluorescence.

QDs as contrast agents

In medical diagnostics, various imaging procedures such as magnetic resonance imaging (MRI) (a non-invasive technique), Ultrasound (non-invasive), computerized tomography scan (CT scan) (invasive technique) and radioactive imaging such as scintigraphy and positron emission tomography (PET) employ contrast agents like paramagnetic iron oxide nanoparticles (MRI), halogenated organic compounds for X-ray, fluoro-deoxy glucose for PET or fluorocarbon filled microbubbles for ultrasound. For in vivo imaging of organs/tissues, preferential accumulation of a contrast agent in sub-lethal doses in the target structure is the desired principle. This could be achieved either through direct injection into the target organ or administering a substance systemically that would “passively” accumulate in the intended tissue owing to its biophysical properties or combine “passive” targeting with molecular recognition and thereby make it “active targeting”. In the latter two methods i.e. passive and active targeting, a long circulation time in blood evading extrusion from the body by defense systems like reticulo-endothelial system is an essential feature to be endowed upon the contrast agent. Relevant studies demonstrated long blood circulation times with colloidal stability for PEGylated particles (PEG of appropriate chain length) (Ballou et al., 2004). If the intended targeting is of “active” mode, receptor ligands should be incorporated into the surface coating. Several studies in recent times have successfully employed QDs (with/ without fluorescence), with optical detection in imaging (Larson et al., 2003; Ballou et al., 2004; Lim et al., 2003; Kim et al., 2003).

Kim et al. (2004) in their studies reported an exciting medical application which involved local administration of QDs as contrast agents intraepidermally (paw of mouse and thigh of pig) and their subsequent uptake by lymphatic vessels passing to the sentinel nodes (in the axilla of the mouse and in the groin of the pig) were imaged. These results reveal that QDs as contrast agents could be well used in surgical procedures (Uren, 2004) with “ease” for the surgeon in locating and delimiting the part of the tissue to be excised and thereby the extent of incision to be made. The only limitation, hitherto, is the paucity of information on the toxicity to tissues. Gao et al. (2004) described the use of multifunctional QD probes for cancer targeting and imaging in living animals. Their cell tracking-imaging study included passive targeting to experimental tumors through systemic administration and also performed active targeting by coupling a tumour specific antibody to the QD probes. They were able to specifically label “nuclei” of cells in culture before they were inoculated in the animals in order to grow tumors. Subsequently, the labeled cells could be tracked and imaged by virtue of their fluorescence.

Toxicity tests on QDs

Studies indicate that QDs cannot be considered as a uniform group of substances and their characteristics, more particularly toxicity depend on multiple factors which include their inherent physico-chemical properties and environmental conditions as well. Toxicity of QDs is reported to be influenced by their size, charge, concentrations, outer coating bioactivity and oxidative, photolytic and mechanical stability (Ron Hardman, 2005). Although QD nanoparticles have proved to be excellent materials for biomedical imaging, drug targeting and in the electronics industries, it is suggestive to investigate the toxic effects of these particles on various tissues like skin. Since the QD core consists of heavy metals, any alteration in their shell or surface coating will lead to the leaching of heavy metal and cause a potential health risk. With the increase in use of QD, the penetration, localization and toxicity of QDs in skin and skin cells became an issue of concern and Zhang et al. (2008) carried out a study on biological interactions of QD in skin and in human epidermal keratinocytes. Results of the study indicate that QD penetration of skin depends on the type, shape of the rigid core and/or size and more essentially on the surface charge. The study reports that of the several types of PEG coated QDs tested QD 621 remains on the surface of the skin and sometimes near hair follicles. All the three PEGylated QDs (QD 621, QD

565 and QD 655) had the same chemical composition with respect to their core and surface coating but showed varying penetration properties. Earlier studies suggest that elastic properties enable the particles to penetrate faster through the epidermis while rigid particles remain on the surface of the upper skin cells (Honeywell-Nguyen et al., 2004). The common route of penetration in skin is through the intercellular spaces between the corneocytes and studies show that the vertical and lateral gaps between corneocytes are 19nm (Van der Merwe et al., 2006). Since the characteristic nail shaped QD 621 employed by Zhang et al. (2008) had an overall size of 39-40 nm and as the surface PEG coating was soft, these particles alone could penetrate and remain lodged with the skin cells' lipid bilayers. On the other hand, spherical QD 565 and elliptical QD 655 which were smaller and "more" regular in shape could well penetrate the skin. The extent of penetration and the process depend on the intercellular lipid structure or hair follicle density (Monteiro-Riviere, 2008). There are some important reports on the penetration behaviour of nanomaterials like TiO_2 and ZnO which are used as key ingredients in sunscreen creams (Gamer et al., 2006; Cross et al., 2007). In this context, the findings of the study by Zhang et al. (2008) throw light on safe biomedical applications of the QD and suggest that their characteristics like core, shell, surface coatings, shape, size and charge should be tested on skin and human epidermal keratinocytes for minimizing dermal toxicity or irritation. Further, if QD are to be used in biological applications, the lowest concentration with lowest toxicity and high fluorescence intensity need to be identified for individual types rather than a general formula.

In recent years, non-heavy metal quantum dots for life science research have been introduced commercially by the Evident Technology (Troy, New York). These new QDs, called T2-MP EviTags^{TM} possess a ternary core consisting of indium, gallium phosphide coated with a metallic plating shell and a natural coating on the outer layer that offers them low toxicity and a wide range of colours into the near infrared.

CORNELL DOTS

An exciting research study was reported in the year 2005 by the researchers of Cornell University. They have created fluorescent nanoparticles by encapsulating fluorescent dyes with a protective silica shell. These particles with possible applications in biological imaging, optical computing, sensors and microarrays such as DNA chips appear to gradually replace QDs because of their greater chemical inertness and reduced cost. Cornell dots, also known as CU dots, are nanoparticles consisting of a core (about 2.2 nm in diameter) constituted by several dye molecules and surrounded by a protective silica shell, making the entire particle about 25 nm in diameter. CU dots are 20-30 times brighter than single dye molecules in solution, resist photobleaching and produce a large assortment of colours. Therefore, Cornell dots could preferentially be used for biological tagging, imaging and optical computing (Wiesner et al., 2005). CU dots fluoresce so brightly that they can be seen through the skin of a mouse. Experimental studies revealed that they accumulate in organs like liver and bladder within hours after injection and could be harmlessly excreted after they perform their function. Since, CU dots are biologically safe, stable and small enough to be easily transported across the body's structures accompanied by harmless excretion, they are being put to use to "light up" cancerous tumors enabling the surgeons to excise them efficiently. PEGylation of CU dots protects them from being recognized as antigens by body's defense system and enables them to find targeted tumors in considerable time. This technology could be used to know the extent of angiogenesis in tumors, cell death, treatment response and metastatic spread to lymph nodes and other organs of the body. High sensitivity and specificity of CU dots as probes in molecular imaging pave the way for early diagnosis with precision by medical practitioners which is vital for effective treatment of patients.

CONCLUSION

A wide array of nanomaterials and nanodevices are being developed through extensive research in laboratories. These research findings should be evaluated in terms of safety, easy administration and detection, efficacy and affordability for the benefit of extensive practical applications in the field of biomedicine. Quantum dots and Cornell dots, which are semiconductor nanocrystal materials, are being synthesized with a variety of protocols depending on their application. Customized quantum dots are also made commercially available by Quantum Dot Corporation and others. Further, need - based clinical research including toxicological studies will unravel the potential applications of these magic materials in the fields of biology and medicine.

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REFERENCES

Abbot A, Cyranoski D (2003). Biology's new dimension. Nature 424: 870-872.

Adleman LM (1994). Molecular computation of solutions of combinatorial problems. Science 266: 1021-1024.
Akerman ME, Chan WC, Laakkonen P, Bhatia SN, Ruoslahti E (2002). Nanocrystal targeting *in vivo*. Proc. Natl. Acad. Sci. USA 99: 12617-12621.
Albrecht-Buehler G (1977a). The phagokinetic tracks of 3T3 cells. Cell 11: 395-404.
Albrecht-Buehler G (1977b). Phagokinetic tracks of 3T3 cells: parallels between the orientation of track segments and of cellular structures which contain actin or tubulin. Cell 12: 333-339.
Albrecht-Buehler G, Lancaster RM (1976). A quantitative description of the extension and retraction of surface protrusions in spreading 3T3 mouse fibroblasts. J. Cell Biol. 71: 370-382.
Aldama J, Wang YA, Peng X (2001). Photochemical instability of CdSe nanocrystals coated by hydrophilic thiols. J. Am. Chem. Soc. 123: 8844-8850.
Ballou B, Lagerholm BC, Ernst LA, Bruchez MP, Waggoner AS (2004). Non invasive imaging of quantum dots in mice. Bioconjugate Chem. 15: 79-86.
Battersby BJ, Lawrie GA, Johnston APR, Trau M (2002). Optical barcoding of colloidal suspensions: applications in genomics, proteomics and drug discovery. Chem. Commun. 14: 1435-1441.
Birge RR (1995). Protein based Computers. Sci. Am. 3: 90-95.
Bruchez MJ, Moronne M, Gin P, Weiss S, Alivisatos AP (1998). Semiconductor nanocrystals as fluorescent biological labels. Science 281: 2013-2016.
Chan WCW, Nie SM (1998). Quantum dot bioconjugates for ultrasensitive nonisotropic detection. Science 281: 2016-2018.
Chang E, Thekkek N, Yu WW, Colvin VL, Drezek R (2006). Evaluation of quantum dot cytotoxicity based on intracellular uptake. Small 12: 1412-1417.
Cheon J, Lee JH (2008). Synergistically integrated nanoparticles as multimodal probes for nanobiotechnology. Acc. Chem. Res. 41: 1630-1640.
Choi Y, Thomas T, Kotlyan A, Islam MT, Baker Jr. JR (2001). Synthesis and functional evaluation of DNA – assembled polyamidoamine dendrimer clusters for cancer cell-specific targeting. Chem. Biol. 12: 3543-3552.
Cross SE, Innes B, Roberts MS, Tsuzuki T, Robertson TA, McCormick P (2007). Human skin penetration of sunscreen nanoparticles: *in-vitro* assessment of a novel micronized zinc oxide formulation. Skin Pharmacol. Physiol. 20: 148-154.
Dahan M, Laurence T, Pinaud F, Chemla DS, Alivisatos AP, Sauer M, Weiss S (2001). Time-gated biological imaging by use of colloidal quantum dots. Opt. Lett. 26: 825-827.
Dahan M, Levi S, Luccardini C, Rostaing P, Riveau B, Triller A (2003). Diffusion dynamics of glycine receptors revealed by single-quantum dot tracking. Science 302: 442-445.
Demers LM, Mirkin CA, Mucic RC, Reynolds RA, Letsinger RL, Elghanian R, Viswanadham G (2000). A fluorescence-based method for determining the surface coverage and hybridization efficiency of thiol-capped oligonucleotides bound to gold thin films and nanoparticles. Anal. Chem. 72: 5535-5541.
Derfus AM, Chan WCW and Bhatia SN (2004). Probing the cytotoxicity of semiconductor quantum dots. Nano. Lett. 4: 11-18.
Dubertret B, Skourides P, Norris DJ, Noireaux V, Brivanlou AH, Libehaber A (2002). *In vivo* imaging of quantum dots encapsulated in phospholipids micelles. Science 298: 1759-1762.
Duncan R, Vicent MJ, Greco F, Nicholson RI (2005). Polymer-drug conjugates: towards a novel approach for the treatment of endocrine-related cancer. Endocrine-Related Cancer 12: S189-S199.
Gamer AO, Leibold E, Van Ravenzwaay B (2006). The *in vitro* absorption of microfine zinc oxide and titanium dioxide through porcine skin. Toxicol. *In Vitro* 20: 301-307.
Gao J, Gu H, Xu B (2009). Multifunctional magnetic nanoparticles: Design, Synthesis and Biomedical applications. Acc. Chem. Res. 42(8): 1097-1107.
Gao JH, Li L, Ho PL, Mak GC, Gu HW, Xu B (2006). Combining fluorescent probes and biofunctional magnetic nanoparticles for rapid detection of bacteria in human blood. Adv. Mater. 18: 3145-3148.
Gao JH, Liang GL, Cheung JS, Pan Y, Kuang Y, Zhao F, Zhang B, Zhang XX, Wu EX, Xu B (2008a). Multifunctional yolk-shell nanoparticles: A potential MRI contrast and anticancer agent. J. Am. Chem. Soc. 130: 11828-11833.
Gao JH, Liang GL, Zhang B, Kuang Y, Zhang XX, Xu B (2007). FePt@CoS_2 yolk-shell nanocrystals as a potent agent to kill HeLa cells. J. Am. Chem. Soc. 129: 1428-1433.
Gao JH, Zhang B, Zhang XX, Xu B (2006a). Magnetic dipolar interaction induced self-assembly affords wires of hollow nanocrystals of cobalt selenide. Angew. Chem. Int. Ed. 45: 1220-1223.
Gao JH, Zhang W, Huang PB, Zhang B, Zhang XX, Xu B (2008). Intracellular spatial control of fluorescent magnetic nanoparticles. J. Am. Chem. Soc. 130: 3710-3711.
Gao X, Nie S (2001). Biologists join the dots. Nature 13: 450-452.
Gao X, Cui Y, Levenson RM, Chung LW, Nie S. (2004). *In vivo* cancer targeting and imaging with semiconductor quantum dots. Nat. Biotechnol. 22: 969-976.
Geiger B, Volberg T (1994). Conjugation of fluorescent dyes to antibodies . Cell Biology – A Laboratory Hand Book ed JE Celis (San Diego, CS: Academic) pp. 387-393.
Ghasemi Y, Peymani P, Afifi S (2009). Quantum dot: magic nanoparticle for imaging, detection and targeting. Acta Biomed. 80: 156-165.
Gu HW, Ho PL, Tsang KWT, Wang L, Xu B (2003a). Using biofunctional magnetic nanoparticles to capture vancomycin-resistant enterococci and other gram positive bacteria at ultralow concentration. J. Am. Chem. Soc. 125: 15702-15703.
Gu HW, Ho PL, Tsang KWT, Yu CW, Xu B (2003b). Using biofunctional magnetic nanoparticles to capture gram negative bacteria at ultra-low concentration. Chem. Commun. pp. 1966-1967.
Gu HW, Zheng RK, Liu H, Zhang XX, Xu B (2005). Direct synthesis of bimodal nanosponge based on FePt and ZnS. Small 1: 402-406.
Gupta AK, Gupta M (2005). Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. Biomaterials 26: 3995-4021.
Han MY, Gao XH, Su JZ, Nie S (2001). Quantum-dot-tagged microbeads for multiplexed optical coding of biomolecules. Nat. Biotechnol. 19: 631-635.
Hanaki K, Momo A, Oku T, Komoto A, Maenosono S, Yamaguchi Y, Yamamoto K (2003). Semiconductor quantum dot/albumin complex is a long-life and highly photostable endosome marker. Biochem. Biophys. Res. Commun. 302: 496-501.
Hanes J, Cleland JL, Langer R (1997). New advances in microsphere-based single dose vaccines. Adv. Drug Del. Rev. 28: 97-119.
Hartgerink JD, Beniash E, Stupp SI (2001). Self-assembly and mineralization of peptide- amphiphile nanofibers. Science 294: 1684-1688.
Herzog M, Draeger A, Ehler E, Small JV (1994). Immunofluorescence microscopy of the cytoskeleton: double and triple immuno-fluorescence. Cell Biology – A Laboratory Handbook ed JE Celis (San Diego, CA/Academic) pp. 355-360.
Hines MA, Guyot-Sionnest P (1996). Synthesis and characterization of strongly luminescing ZnS-capped CdSe nanocrystals. J. Phys. Chem. 100: 468-471.
Honeywell-Nguyen PL, Gooris GS, Bouwstra JA (2004). Quantitative assessment of the transport of elastic and rigid vesicle components and a model drug from these vesicle formulations into human skin *in vivo*. J. Invest. Dermatol. 123: 902-910.
Hu EL, Shaw DT (1999). Synthesis and assembly. In: Siegel RW, Hu EL, Roco MC, eds. Nanoscience Structure and Technology. National Science and Technology Council.
Imae Y, Atsumi T (1989). Na^+ driven bacterial flagellar motors: a mini-review. J. Bioenerg Biomembr. 21: 705-716.
Jaiswal JK, Mattoussi H, Mauro JM, Simon SM (2003). Long-term multiple colour imaging of live cells using quantum dot bioconjugates. Nat. Biotechnol. 21: 47-51.
Jiang J, Gu HW, Shao HL, Devlin B, Papaetthymiou GC, Ying JY (2008). Bifunctional Fe_3O_4-Ag heterodimer nanoparticles for two-photon fluorescence imaging and magnetic manipulation. Adv. Mater. 20: 4403-4407.
Karak N, Maiti S (1997) Dendritic Polymers: a class of novel material. J. Polym. Mater. 14: 105.
Kim H, Achermann M, Balet LP, Hollingsworth JA, Klimov VI (2005).

Synthesis and characterization of Co/CdSe core/shell nanocomposites: Bifunctional magnetic-optical nanocrystals. J. Am. Chem. Soc. 127: 544-546.
Kim S, Fisher B, Eisler HJ, Bawendi M (2003). Type – II quantum dots: CdTe/CdSe (Core/Shell) and CdSe/ZnTe (Core/Shell) hetero-structures. J. Am. Chem. Soc. 125: 11466-11467.
Kim S, Lim YT, Soltesz EG, De Grand AM, Lee J, Nakayama A, Parker JA, Mihaljevic T, Laurence RG, Dor DM, Cohn LH, Bawendi MG, Frangioni JV (2004). Near-infrared fluorescence type II quantum dots for sentinel lymph node mapping. Nat. Biotechnol. 22: 93-97.
Kloepfer JA, Mielke RE, Wong MS, Nealson KH, Stucky G, Nadeau JL (2003). Quantum dots as strain and metabolism specific microbiological labels. Appl. Environ. Microbiol. 69: 4205-4213.
Kralj M, Pavelic K (2003). Medicine on a small scale. EMBO Reports 4: 1008-1012.
La VD, Lynn DM, Langer R (2002). Moving smaller in drug discovery and delivery. Nat. Rev. Drug Discov. 1: 77-84.
Larson DR, Zipfel WR, Williams RM, Clark SW, Bruchez MP, Wise FW, Webb WW (2003). Water-soluble quantum dots for multiphoton fluorescence imaging *in vivo*. Science 300: 1434-1436.
Laus S, Ruloff R, Toth E, Merbach AE (2003). GdIII Complexes with fast water exchange and high thermodynamic stability: potential building blocks for high-relaxivity MRI contrast agents. Chem. 9: 3555-3566.
Leroux JC (2007). Injectable nanocarriers for biodetoxification. Nat. Nanotechnol. 2: 679-684.
Lidke DS, Nagy P, Heintzmann R, Jovin DJ, Post JN, Grecco HE (2004). Quantum dot ligands provide new insights into erbB/HER receptor-mediated signal transduction. Nat. Biotechnol. 22: 198-203.
Lim YT, Kim S, Nakayama A, Stott NE, Bawendi MF, Frangioni JV (2003). Selection of quantum dot wavelengths for biomedical assays and imaging. Mol. Imaging 2: 50-64.
Lockman PR, Mumper RJ, Khan MA, Allen DD (2002). Nanoparticle technology for drug delivery across the blood-brain barrier. Drug Dev. Ind. Pharm. 28: 1-13.
Mansoori GA, Mohazzabi P, McCormack P, Jabbari S (2007). Nanotechnology in cancer prevention, detection and treatment: bright future lies ahead. World Review of Science, Technol. Sustain. Dev. 4: 2-3.
Mattheakis LC, Dias JM, Choi YJ, Gong J, Bruchez MP, Liu J, Wang E (2004). Optical coding of mammalian cells using semiconductor quantum dots. Anal. Biochem. 327(2): 200-208.
Mitchell GP, Mirkin CA, Letsinger RL (1999). Programmed assembly of DNA functionalized quantum dots. J. Am. Chem. Soc. 121: 8122-8123.
Miyawaki A, Sawano A, Kogure T (2003). Lighting up cells: labeling proteins with fluorophores. Nat. Cell Biol. 5S: 1-7.
Monteiro-Riviere NA (2008). In: Zhai H, Wilhelm KP, Maibach HI (Eds.).
Murray CB, Norris DJ, Bawendi MG (1993). Synthesis and Characterization of Nearly Monodisperse Cde (e = S, Se, Te) Semiconductor Nanocrystallites. J. Am. Chem. Soc. 115: 8706-8715.
Ness JM, Akhtar RS, Latham CB, Roth KA (2003). Combined tyramide signal amplification and quantum dots for sensitive and photostable immunofluorescence detection. J. Histochem. Cytochem. 51: 981-987.
Noji H, Yasuda R, Yoshida M, Kinosita K (1997). Direct observation of the rotation of F-1-ATPase. Nature 386: 299-302.
Obeso JL, Auerbach R (1984). A new microtechnique for quantitating cell movement *in vitro* using polystyrene bead monolayers. J. Immunol. Methods 70: 141-152.
Osborn M (1994). Immunofluorescence microscopy of cultured cells. Cell Biology – A Laboratory Handbook ed JE Celis (San Diego, CA/Academic) pp. 347-54.
Parak WJ, Pellegrino T, Plank C (2005). Labelling of cells with quantum dots. Nanotechnol. 16: R9-R25.
Pathak S, Choi SK, Amheim N, Thompson ME (2001). Hydroxylated quantum dots as luminescent probes for in situ hybridization. J. Am. Chem. Soc. 123: 4103-4104.
Pawley JB, Centonze VE (1994). Practical laser-scanning confocal light microscopy: obtaining optimal performance from your instrument. Cell Biology - A Laboratory Hand Book ed JE Celis (San Diego, CS: Academic) pp. 44-64.
Peng S, Sun SH (2007). Synthesis and Characterization of mono-disperse hollow Fe_3O_4 nanoparticles. Angew. Chem. Int. Ed. 46: 4155-4158.
Peng ZA, Peng XG (2001). Formation of high quality CdTe, CdSe, and CdS nanocrystals using CdO as precursor. J. Am. Chem. Soc. 123: 183-184.
Pinaud F, King D, Moore HP, Weiss S (2004). Bioactivation and cell targeting of semiconductor CsSe/ZnS nanocrystals with phytochelatin-related peptides. J. Am. Chem. Soc. 126: 6115-6123.
Plank C, Oberhauser B, Mechtler K, Koch C, Wagner E (1994). The influence of endosome-disruptive peptides on gene transfer using synthetic virus-like gene transfer systems. J. Biol. Chem. 269: 12918-12924.
Plank C, Zauner W, Wagner E (1998). Application of membrane-active peptides for drug and gene delivery across cellular membranes. Adv. Drug Deliv. Rev. 34: 21-35.
Rikans LE, Yamano T (2000). Mechanisms of cadmium-mediated acute hepatotoxicity. J. Biochem. Mol. Toxicol. 14: 110-117
Ron H (2005). A Toxicological review of Quantum Dots: Toxicity depends on physico-chemical and environmental factos. Environ. Health Perspect. 114(2): 165-172.
Rosenthal SJ, Tomlinson A, Adkins EM (2002). Targeting cell surface receptors with ligand-conjugated nanocrystals. J. Am. Chem. Soc. 124: 4586-4594.
Safarik I, Safarikova M (2004). Magnetic techniques for the isolation and purification of proteins and peptides. Biomagn. Res. Technol. 2: 7.
Saiyeed Z, Telang S, Ramchand C (2003). Application of magnetic techniques in the field of drug discovery and biomedicine. Biomagn. Res. Technol. 1: 2.
Schnur JM (1993). Lipid tubules: a paradigm for molecularly engineering structures. Sci. 262: 1669-1676.
Schnur JM, Price R, Rudolph AS (1994). Biologically engineered microstructures: controlled release applications. J. Control. Release 28: 3-13.
Silva A, Antonio T, Chung MC, Castro FF, Guido RVC, Ferreira EI (2001). Advances in Prodrug design. Mini Rev. Med. Chem. 5(10): 893-914.
Silva GA (2004). Introduction to Nanotechnology and its Applications to Medicine. Surg. Neuro. 61: 216-220.
Skaff H, Emrick T (2003). The use of 4-substituted pyridines to afford amphiphilic, pegylated cadmium selenide nanoparticles. Chem. Commun. 52: 3.
Skrabalak SE, Chen JY, Sun YG, Lu XM, Au L, Cobley CM, Xia YN (2008). Gold nanocages: Synthesis, properties and applications. Acc. Chem. Res. 41: 1587-1959.
Sonawane ND, Szoka FC, Verkman AS (2003). Chloride accumulation and swelling in endosomes enhances DNA transfer by polyamine-DNA polyplexes. J. Biol. Chem. 278: 44826-44831.
Stephens DJ, Allan VJ (2003). Light microscopy techniques for live cell imaging. Sci. 300: 82-86.
Stupp SI, Ciegler GW (1992). Organoapatites: materials for artificial bone. I. Synthesis and microstructure. J. Biomed. Mater. Res. 26: 169-183.
Stupp SI, Mejicano GC, Hanson JA (1993). Organoapatites: materials for artificial bone. II. Hardening reactions and properties. J. Biomed. Mater. Res. 27: 289-299.
Sukhanova A, Devy M, Venteo L, Kaplan H, Artemyev M, Oleinikov V, Klinov D, Pluot M, Cohen JH, Nabiev I (2004). Biocompatible fluorescent nanocrystals for immunolabeling of membrane proteins and cells. Anal. Biochem. 324: 60-67.
Sukhanova A, Venteo L, Devy J, Artemyev M, Oleinikov V, Pluot M, Nabiev I (2002). Highly stable fluorescent nanocrystals as a novel class of labels for immunohistochemical analysis of paraffin-embedded tissue sections. Lab. Invest. 82: 1259-1261.
Takano H, Sul JY, Mazzanti Ml, Doyle RT, Haydon PG, Porter MD (2002). Micropatterned substrates: approach to probing intercellular communication pathways. Anal. Chem. 74: 4640-4646.
Tombolini R, Jansson JK (1998). Monitoring of GFP-tagged bacterial cells. Bioluminiscence Methods and Protocols ed RA La Rossa (Totowa, NJ: Humana Press) pp. 285-98.
Tan J, Wang B, Zhu L (2006). Quantum Dots: A novel tool to

discovery. Pak. J. Biol. Sci. 9(5): 917-922.
Torchilin V, Weissig V (2003). Liposomes: a practical approach. The Practical Approach Series #264, Oxford Univ. Press, Aug. 7, Oxford, GB.
Uren RF (2004). Cancer surgery joins the dots. Nat. Biotechnol. 22: 38-39.
Van der MD, Brooks JD, Gehring R, Baynes RE, Monteiro_Riviere NA, Riviere JE (2006). A physiologically based pharmacokinetic model of organophosphate dermal absorption. Toxicol. Sci. 89: 188-204.
Wagner E, Plank C, Zatloukal K, Cotton M, Birnstiel ML (1992). Influenza virus hemagglutinin HA-2 N-terminal fusogenic peptides augment gene transfer by transferring-polylysine-DNA complexes: toward a synthetic virus-like gene-transfer vehicle. Proc. Nat. Acad. Sci. USA 89: 7934-7938.
Wang YA, Li JJ, Chen HY, Peng XG (2002). Stabilization of inorganic nanocrystals by organic dendrons. J. Am. Chem. Soc. 124: 2293-2298.
Weijer CJ (2003). Visualizing signals moving in cells. Sci. 300: 96-100.
Weisner, Hooisweng Ow, Daniel R. Larson, Mamta Srivastava, Barbara A. Baird, Watt WW (2005). “Bright and stable Core-shell Fluorescent Silica Nanoparticles”. Nano Lett. 5(1).
Winter JO, Liu TY, Korgel BA, Schmidt CE (2001). Recognition molecule directed interfacing between semiconductor quantum dots and nerve cells. Adv. Mater. 13: 1673-1677.
Wu X, Liu H, Liu J, Haley KN, Treadway, JA, Larson JP, Ge N, Peale F, Bruchez MP (2003). Immunofluorescent labeling of cancer marker Her2 and other cellular targets with semiconductor quantum dots. Nat. Biotechnol. 21: 41-46.
Xu CJ, Xie J, Ho D, Wang C, Kohler N, Walsh EG, Morgan JR, Chin YE, Sun SH (2008). Au-Fe_3O_4 dumbbell nanoparticles as dual-functional probes. Angew. Chem. Int. Ed. 47: 173-176.
Xu CJ, Xu KM, Gu HW, Zheng RK, Liu H, Zhang XX, Guo ZH, Xu B (2004). Dopamine as a robust anchor to immobilize functional molecules on the iron oxide shell of magnetic nanoparticles. J. Am. Chem. Soc. 126: 9938-9939.
Xu CJ, Xu KM, Gu HW, Zhong XF, Liu H, Guo ZH, Zheng RK, Zhang XX, Xu B (2004b). Nitrilotriacetic acid-modified magnetic nanoparticles as a general agent t bind histidine-tagged proteins. J. Am. Chem. Soc. 126: 3392-3393.
Yin YD, Rioux RM, Erdonmez CK, Hughes S, Somorjai GA, Alivisatos AP (2004). Formation of hollow nanocrystals through the nanoscale kirkendall effect. Sci. 304: 711-714.
Yu H, Chen M, Rice PM, Wang SX, White RL, Sun SH (2005). Dumbbell-like bifunctional Au- Fe_3O_4 nanoparticles. Nano Lett. 5: 379-382.
Zhang A, Zhang B, Wachtersbach E, Schmidt M, Schluter AD (2003). Efficient synthesis of high molar mass first to fourth generation distributed dendronized polymers by the macromonomer approach. Chem. Eur. J. 9(24): 6083-6092.
Zhang C, Ma H, Ding Y, Jin L, Chen D, Nie S (2000). Quantum dot-labeled trichosanthin. Analyst 125: 1029-1031.
Zhang LW, Yu WW, Colvin VL, Monteiro RNA (2008). Biological interactions of Quantum Dot nanoparticles in skin and in human epidermal keratinocytes. Toxicol. Appl. Pharmacol. 228: 200-211.