ZnO quantum dots for biomedical applications

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ABSTRACT

Quantum dots (QDs), highly luminescent semiconductor nanocrystals, have found extensive applications in different fields, ranging from optoelectronic to bio-imaging. Numerous applications are emerging daily. Among these, ZnO QDs have higher biological significance because of their relative non-toxicity. The primary aim of this review is to overview the literature based on the biological applications of ZnO QDs, including gene therapy, drug delivery, optical imaging, allergen and antigen detection, cancer cell sensing, antibacterial agents and DNA detection. The luminescent properties of ZnO nanoparticles have attracted considerable attention for numerous applications like ultraviolet light emitting devices, flat panel displays, as low voltage phosphors and biosensing devices. The review throws light on the developments in fabrication techniques of nanometer-sized, water-dispersible, bio-compatible and stable ZnO QDs in aqueous medium for biological applications, including employing organic ligands, coating nanoparticles with inorganic shells, doping with a suitable element and capping nanoparticles surfaces with polymers. The low toxicity of ZnO and its high natural abundance make it a good alternative to cadmium based II-VI semiconductors, which cause toxicity via photoinduced reactive oxygen species (ROS) generation. Copyright © 2013 VBRI press.

Keywords: Quantum dots; photoluminescence; imaging; detection; biolabels; surface modification.

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Rita Kakkar, after obtaining a PhD degree in Physical Chemistry from the University of Delhi, undertook research on various topics. She has been teaching physical chemistry at the University of Delhi for the past over three decades. Her main research interests are in Computational Chemistry and related fields. She heads a large research group, which is carrying out computational and experimental studies on catalysis by nanomaterials and by enzymes. Her research on nanoscale materials also includes theoretical and experimental studies on quantum dots and their size-dependent properties for use as semiconductor devices and sensors. Professor Kakkar has over 80 research publications in international journals. She has successfully supervised the work of 33 PhD and 7 MPhil students. She has delivered invited several talks at scientific conferences. She has acted as an International Advisory Member for several conferences on computational chemistry. She regularly reviews manuscripts for many international journals, including those published by the American Chemical Society, Royal Society of Chemistry and Elsevier.

Introduction

Quantum dots are a special class of materials, which are nanocrystals of inorganic semiconductors composed of atoms of periodic groups of II-VI, III-V, or IV-VI. The term quantum dots was first coined by Mark A. Reed in 1988 and developed in the early 1980s by Louis Brus at Bell laboratories **[1],** along with Alexander Efros and Alexei I. Ekimov **[2]** of the A. F. Ioffe Physical Technical Institute. Quantum dots (QDs) exhibit unique properties which include broad excitation and narrow size-tunable emission spectra (usually 20–40 nm full width at half maximum intensity), negligible photobleaching, and high photochemical stability **[3, 4]**. During the past few decades, the work on semiconductor nanocrystals has immensely improved due to their remarkable optical, electrical and catalytic properties. The surface chemistry behavior of luminescent quantum dots is of immense interest as it has strengthened the development of multiple probes based on linked recognition molecules, such as peptides, nucleic acids or small-molecule ligands. These highly luminescent semiconductor nanocrystals have found extensive applications in different fields, ranging from optoelectronic to bio-imaging.

This review analyses the biomedical applications of ZnO quantum dots. The scope of this article is focused on the application of ZnO quantum dots in various fields such as gene therapy, drug delivery, optical imaging, allergen

and antigen detection, cancer cell sensing, antibacterial agents and DNA detection.

Quantum dots in biology

During the past decade, the photoluminescent semiconductor quantum dots have received considerable attention as biological labels and fluorescent biosensors (**Fig. 1**). Such quantum dots possess several advantages in comparison with the organic dyes and fluorescent proteins, such as unique optical and electronic properties, size tunable light emission, improved signal brightness, resistance against photobleaching and simultaneous excitation of multiple fluorescence colors. In contrast, organic dyes do not show such tunability. A very broad absorption spectrum enables the excitation of a mixture of quantum dots of different emission colors with a single excitation wavelength, while different excitation wavelengths are needed to excite each organic dye in a solution containing several dyes due to their narrow absorption spectra.
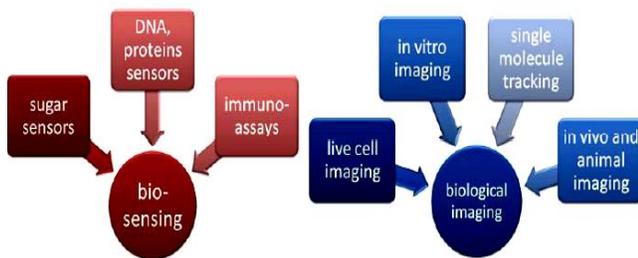

Fig. 1. Examples of QDs' bioanalytical and biomedical applications **[5].**

Nanocrystals of CdSe and CdTe **[6]** and CdTe/ZnTe **[7]** are reported to be the most popular biolabels in imaging and detection. Recent works have highlighted the acute toxicity of Cd based core materials on biological systems **[8, 9]** due to the release of toxic heavy metal ions from the core of the material, and formation of reactive oxygen species (ROS). The toxicity of Cd-related compound is an urgent concern as it limits the use of these visible or near IR emitting nanocrystals, especially for applications directly related to human health. Therefore, searching for non-toxic substitutes is the most challenging aspect of working with these materials in the biomedical field.

The ZnO quantum dot: An alternative and promising quantum dot

A promising alternative to Cd-free QDs is ZnO. Different aspects of Zn-based quantum dots ranging from the materials aspects in terms of nanocrystal shapes (wire, rod, cone and spherical), lattice structures, doping and surface modifications **[10-13]** to optoelectronic aspects such as luminescence properties, banding energy and band gap studies **[14, 15]** have been studied recently. Due to its wide bandgap (3.37 eV) and high exciton binding energy (60 meV), the importance of zinc oxide (ZnO), among other metal oxides has been increasing tremendously in various fields. It is one of the versatile materials with extensive applications in medicines, pigments, photo-catalysts, solar cells, chemical sensors, piezoelectric transducers,

transparent electrodes, electroluminescent devices and ultraviolet laser diodes. The ZnO quantum dot is an environmentally friendly material and has great potential application in the field of biology such as bio-imaging and cancer detection. Low material cost and excellent biocompatibility makes the ZnO QD an excellent candidate for biomedical applications.

Properties of ZnO quantum dots

Quantum confinement

Compared to their bulk counterparts, ZnO nanostructures exhibit novel optical and electronic properties due to the quantum confinement of excitons and phonons in nanostructures **[16-19]**. The phenomenon of quantum confinement arises once the diameter of the particle is of the same magnitude as the wavelength of the electron wave function. Quantum confinement generally results in widening of the band gap, *i.e.* the gap between the conduction band and the valence band, increases as the size of the nanostructure decreases (**Fig. 2**). It is because of the quantum confinement effect that quantum dots of the same material with different sizes exhibit various colors.

ZnO QDs having sizes <3.6 nm experience strong quantum confinement in all the dimensions, *i.e.* movements of electrons and holes are restricted in all three directions. Thus, the density of states for this zero dimension (0D) system will be delta functions. Small spatial correlation between electrons and holes produce a significant change in the optical properties of these quantum dots.
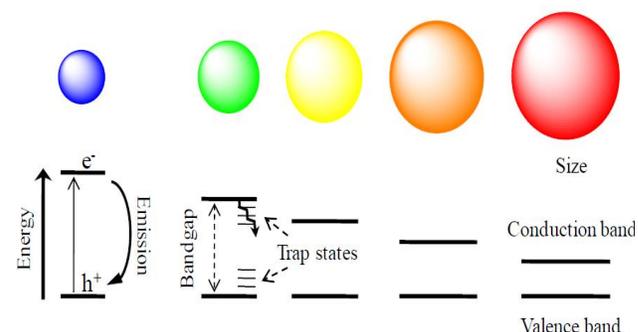

Fig. 2. Illustration of size-tunable quantum dots and creation of the exciton (electron-hole pair) upon photoexcitation followed by radiative recombination (fluorescence emission) or relaxation through trap states **[20]**.

Luminescence properties

As already discussed, ZnO quantum dots have received considerable attention owing to their unusual electronic and optical properties. The optical and electronic properties of semiconductor nanoclusters arise from the interaction between electrons, holes, and their local environments. Quantum dots absorb photons when the excitation energy exceeds their bandgap and after absorbing energy, an electron may jump from the ground state to a higher excited state. The energies associated with such optical absorptions are directly determined by the electronic structure of the material. The excited electron and hole forms an exciton. The electron may recombine with a hole and relax to a lower energy state, ultimately reaching the ground state.

The excess energy resulting from the recombination and relaxation may be either radiative (emit photon) or non-radiative (emit phonons). Radiative relaxation results in spontaneous luminescence from quantum dots. Luminescence may result from band-edge or near band-edge transitions.

For wurtzite ZnO, there are normally two photoluminescent emission bands; one is centred in the UV region and the other in the visible region. The UV emission arises due to photo-generated electron recombination with holes in the valence band or in traps near the valence band (**Fig. 3),** while the mechanism for the second component *i.e.* visible emission (also called deep-level emission) is controversial.
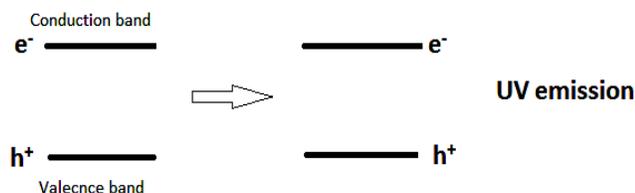

Fig. 3. UV component of ZnO nanoparticles.

Different plausible luminescence mechanisms have been proposed to explain the origin of the visible emission [21]. The two most cited mechanisms, (A) recombination of a shallowly trapped electron with a hole in a deep trap, (B) recombination of an electron in a singly occupied oxygen vacancy with a photo-generated hole in the valence band [22], are shown in **Fig. 4**.
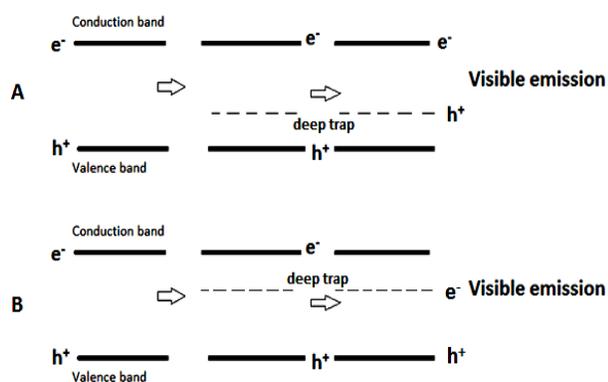

Fig. 4. Visible component of ZnO nanoparticles.

The size of ZnO quantum dots is used to tune the band gap across a major portion of the visible spectrum. A progressive blue shift from 3.43 to 3.65 eV was observed as the size of the ZnO quantum dot decreases [23]. This ability of using "size" as a variable in tailoring the optical properties of the ZnO QDs has made these quantum dots excellent candidates for biosensing applications owing to their unique optical properties.

Surface modification

ZnO QDs are promising alternatives for diagnosis and imaging due to their non-toxicity, thermal and chemical stability, but their aqueous instability has markedly limited their use. Conventional ZnO quantum dots are not very stable in water. This instability is related to their surface

luminescent mechanisms, as water will exchange the organic protecting groups on the ZnO surface attacking the luminescent centers and destroy them rapidly. In recent years, different strategies for their surface modification have been developed for the transfer of hydrophobic ZnO QDs in water with preservation of their luminescent properties. Highly luminescent (visible to the naked eye on UV illumination), transparent, chemically pure, and crystalline ZnO QDs using LP-PLA (liquid-phase pulsed laser ablation) without the aid of any surfactant have been reported [24]. The emission wavelength was tuned by varying the native defect chemistry of ZnO QDs and the laser fluence. These highly luminescent, nontoxic and bio-friendly ZnO QDs have exciting application potential as fluorescent probes in biomedical applications by easily attaching biomolecules to the bare surface of these quantum dots.

Surface effects significantly influence the functionality of semiconductor nanocrystals. Studies on the surface morphology of semiconductor nanoparticles indicate that the irregular morphology and defects affect the luminescent property of ZnO quantum dots. Surface modification of ZnO quantum dots by mercaptoacetic acid (MAA) [25] resulted in a major impact on the luminescence properties of ZnO quantum dots because it can strongly adsorb and etch quantum dots through its mercapto-functional group, thus modifying and reducing the surface defects significantly, which improves the exciton emission peak and luminescence properties by preventing reunion.
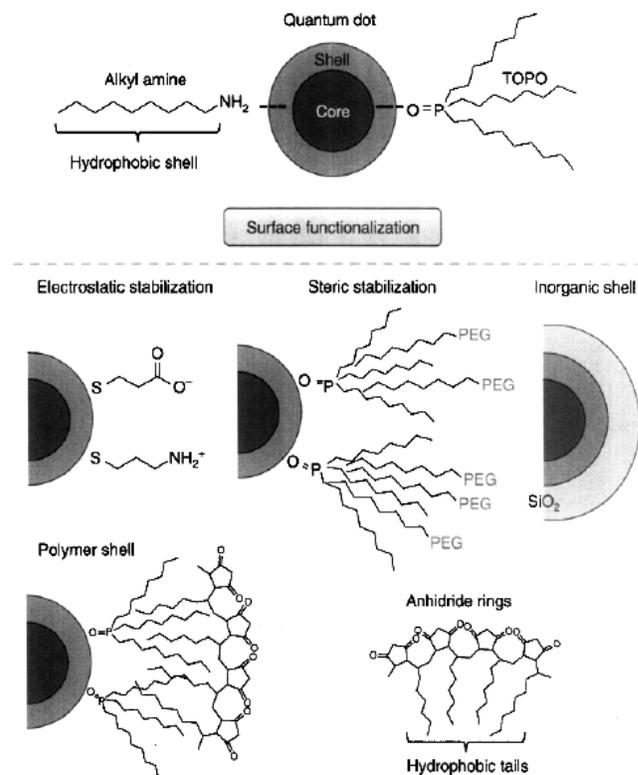

Fig. 5. Surface functionalization of quantum dots using various capping ligands [26].

In MAA modified ZnO QDs, electrons get transferred to the lowest unoccupied orbital of mercaptoacetic acid

through non-radiative transitions from the conduction band and exciton energy, thus decreasing the probability of electronic transition for transfer from the exciton energy back to the valence band, because of the enhancement of non-radiative transition of electrons. This modification results in exciton fluorescence quenching with decreased fluorescence intensity and provides stability and certain ability to resist electrolytes, which is an important reference value for using ZnO QDs as bio-analysis markers.

Passivation with ligands or high band gap semiconductor shells is necessary to reduce surface trap densities, which causes enhancement in the quantum yield and thus increases photostability (**Fig. 5**). Because of its large band gap and large excitation binding energy, ZnO can also be used for new applications in bio-imaging after careful surface modifications. ZnO has high surface-to-volume ratio at the nanometer scale, and hence surface defects play an important role in its properties. An essential surface modification needs to be done for each of the desired applications. In order to protect ZnO nanoparticles and improve their optical properties, polymers, alkoxysilanes, oleic acid, etc. are mixed with ZnO QDs or ZnO surfaces are passivated with these surface modifying agents to produce various nanocomposites. These ligands have been used to stabilize the ZnO QDs by coordinating with the metal atoms on the ZnO surface. The subsequent section will highlight how the surface modification of ZnO quantum dots brings about drastic changes in their luminescent properties, quantum yields and photostability.

Polymer-capped ZnO quantum dots

The stabilization of the photophysical properties of the core can be effectively achieved by coating ZnO QDs with a polymer shell. Ultrastable (ZnO) polymer core–shell nanoparticles with core diameter of 2.1 nm have been synthesized by co-polymerization of these nanoparticles with methyl-methacrylate [27]. A similar strategy was proposed to prepare water-stable poly(methacrylic acid)(PMMA)-capped ZnO QDs by co-polymerization with methacrylic acid to produce ZnO@PMMA microspheres (about 150 nm in diameter), exhibiting blue fluorescence with photoluminescence quantum yield (PL QY) = 22% [28]. Small-sized ZnO@polymer core-shell nanoparticles (diameters of *ca.* 3-4 nm) were reported in 2008 for labeling live cells [29]. Small-sized 2-(dimethylaminoethyl) ethyl methacrylate (DMAEMA) coated ZnO quantum dots (diameter *ca.* 4 nm) exhibiting strong yellow luminescence with PL QY of 21% have been reported [30]. Such small size nanoprobes of ZnO have been used for cell imaging, and are very critical for successful *in vivo* application, since large-sized probes are not suitable for the biological and medical fields, especially for labeling functional subcellular or proteins as they significantly reduce biostability, diffusion and circulation processes, and increase undesired non-specific binding.

A different kind of polymer (biodegradable) that can also be used to stabilize the photophysical core of ZnO has been proposed [31]. Hollow nanospheres of chitosan-ZnO nanoparticles (CS-ZnO NPs) consisting of a cationic polymer CS and an anionic monomer acrylic acid (AA) with an average diameter of *ca.* 150 nm exhibiting strong

blue-emission, centered at 440 nm were prepared. Therefore, the CS-ZnO NPs with tunable and bright fluorescence were expected to be simultaneously used as carriers for guest materials and for biological fluorescent labeling. Because of their inherent biocompatibility and biodegradability, such materials (CS-ZnO NPs) have many potential applications in bio-medicine, including tissue engineering, drug delivery and bio-sensors. For example, Shukla *et al.* [32] have used core-shell nanocomposites based on zinc oxide encapsulated chitosan-*graft*-poly(vinyl alcohol) (ZnO/CHIT-g-PVAL) for glucose sensing. The authors propose that the biosensor system could also be used for the effective determination of cholesterol, triglycerides, etc. in micro/nano molar concentrations.

Siloxane and poly(amidoamine) capped ZnO quantum dots

Organosilanes are highly interesting candidates for surface modification and stabilization of ZnO nanocrystals in order to inhibit decomposition in aqueous media. Owing to the ability of silane molecules to form covalent siloxane bonds with the metal oxide surface, these molecules are found to create a shielding barrier [33, 34] of cross-linked silanes (polysiloxanes) that protect the nanocrystal at the core. ZnO quantum dots were modified with 3-amminopropyltrimethoxysilane, where the ammine groups at the periphery contribute to the stability of these quantum dots [35]. In order to improve the PL intensity and to red-shift the fluorescence emission peaks, a second capping (SiO_2 or TiO_2) was introduced at the periphery of ZnO@APTES nanocrystals. A strong fluorescence was observed in the cell walls of the vascular cylinder when mungbeans seeds were germinated in the presence of these ZnO colloidal dispersions [36]. These observations indicate a good uptake of the nanocrystals and their great potential in bio-imaging studies.

Highly luminescent water-soluble ZnO quantum dots were synthesized by covalent attachment of PAMAM (poly(amidoamine)) dendrons having the siloxane group at the focal point [37]. PAMAM dendrimers have been significantly used as carriers for enhancing bioavailability of drugs and nanoparticles due to their branched nature, high water affinity and multivalent surface groups, providing easier attachment of surface moieties. The PL QY of ZnO@PAMAM reached 59% after 20 days, probably due to a surface-ordering of the siloxane-capping, which results in ZnO QDs with more efficiently protected luminescent centers from water attack. These highly luminescent ZnO QDs were successfully used for imaging of the gram positive bacteria *staphylococcus aureus* and the biocompatibility of these quantum dots was markedly improved compared to Cd-based ones, as growth inhibition tests showed that these dots could be used with concentration up to 1 mM without altering the cell growth.

Carboxymethyl β-cyclodextrin capped ZnO quantum dots

In an alternative method of surface passivation of ZnO quantum dots, highly stable and water soluble ZnO/MgO nanocrystals were prepared by capping these nanocrystals with carboxymethyl β–cyclodextrin (CMCD) cavities [38]. Capping the nanocrystals by cyclodextrin cavities renders the nanocrystal surface hydrophilic due to the existence of

hydroxyl groups on the rim of the cyclodextrin cavity [39]. The surface-anchored cyclodextrin was found to retain its host capabilities for inclusion of small hydrophobic molecules. Herein, fluorescence resonance energy transfer (FRET) occurs from the visible light emitting ZnO/MgO nanocrystals to Nile Red in the noncovalent supramolecular CMCD ZnO/MgO-Nile Red assembly, showing ZnO/MgO QDs to be excellent donors in FRET to Nile Red. The Nile Red emission following resonance energy transfer exhibits a pronounced thermochromic shift (linear blue shift with increasing temperature). Thus, the CMCD-capped ZnO/MgO quantum dots Nile Red assembly could be used as thermometers in aqueous solutions.

Phospholipid-micelle-encapsulated ZnO quantum dots

Successful encapsulation of ZnO nanocrystals within the nonpolar core of phospholipid micelles has been reported [40]. Due to their specific properties, such as low critical micelle concentration and high aqueous stability, such micelles act as stabilizers of nanoparticles and also make them capable of site-specific drug delivery. These micelle encapsulated zinc oxide (ZnO) optical probes ensure longer cell viability and ultimately longer imaging times, and they are ideally suited for bioimaging applications due to accurate selection of the input wavelength, photobleaching resistance and no heat dissipation into the cells. The successful internalization of ZnO-FA nanoparticles in live cells further highlights the great potential of these micelles encapsulated ZnO nanoprobes for photochemical interactions inside the cells, such as photodynamic therapy.

Doped ZnO quantum dots

Doping with suitable elements is an effective approach to modify the electronic, optical and magnetic properties of semiconductor nanocrystals [41, 42]. Rare-earth (RE) elements, as dopants of semiconductors, have a great possibility to efficiently modulate the emission in the visible range due to their unique optical properties and excellent qualification to be radiative centers [43]. Recently, the luminescence properties of ZnO nanostructures doped with various rare earth like Ce [44], Eu [45], Dy [46, 47], Tb [48], Yb and Tm [49] have been widely studied, as they have distinct advantages over heavy metal-containing QDs. ZnO quantum dots with high luminescent properties can be used in many applications such as cell imaging, light-emitting diodes, and anti-counterfeiting purposes. Gd-doped ZnO QDs through surface modification with *N*-(2-aminoethyl) aminopropyltrimethoxysilane [50] have been reported. These Gd doped ZnO QDs were successfully used for the fluorescence imaging of HeLa cells, as the MTT cell proliferation assay indicated such dots to be nontoxic up to 1 mM.

In another work, luminomagnetic (luminescent and magnetic, simultaneously) Fe-doped ZnO nanoparticles, which seemed to possess the relevant properties like small size, luminescence and good magnetism [51], were reported. The surface of the luminomagnetic nanoparticles was modified with *N*-(2-aminoethyly) aminopropyl trimethoxysilane and next conjugated with folic acid using EDC and *N*-hydroxysuccinimide. Because folate receptors

are over-expressed on numerous human cells (breast, ovaries, lungs, kidneys), the folic acid conjugated ZnO:Fe luminomagnets have great potential for various biomedical applications. Thus, surface modification with folic acid makes these doped ZnO QDs feasible nanocarriers for bio imaging applications by combining the specificity of folate receptors on cancer cells with the unique optical and magnetic properties of ZnO nanoparticles in order to develop biocompatible molecular imaging agents, drug delivery systems, and hyperthermia agents.

Biological application of ZnO quantum dots

This section will highlight the biological applications of ZnO quantum dots in various fields.

Table 1. Comparative study of the features of nanoparticle vectors with regard to conventional viral and non-viral vector [52].

Features	Viral-vectors	Non-viral vectors	Nanoparticle vectors
Efficiency	High	Low	Can be high
Synthesis and modification	Function determined by viral structures and not easily modified	Hard to incorporate multiple functions	Easy to incorporate different functions on a single particle
Immunogenicity	Elicit strong immune response	Can be controlled	Can be controlled
Size	Size restricted (30 - 100 mm)	Individual dendrimers (< 2 nm) polymers (> 50 nm)	Size tunable from 1 nm to 200 nm

Polycation-capped ZnO quantum dots: A new type of transgenic vector

Gene therapy is defined as delivery of functional genes to target cells for achieving therapeutic effects. It is considered as a potential medical revolution. The application of gene therapy in the suppression or replacement of malfunctioning genes holds great promise for the cure of a number of disease at the genetic level. Gene delivery systems are categorized as viral and non-viral based. The viral gene delivery system shows a high transfection yield. However, a number of disadvantages, such as oncogenic effects and immunogenicity lead to the development of non-viral gene therapy, an efficient safe vector for gene therapy. It has advantages, such as low host immunogenicity and relatively simple production, forming stable complexes with plasmid, and enhancing cytocompatibility. A new gene delivery system based on various nanoparticulate approaches like cationic lipids, cationic polymers, gold nanoparticles, magnetic nanoparticles, quantum dots, silica nanoparticles, fullerenes, carbon nanotubes, and supramolecular systems [52] has been reported. **Table 1** demonstrates the comparison of various features of nanoparticle vectors with regard to conventional viral and non-viral vector in gene-delivery systems.

Recent work on surface modification of QDs has led to the development of a new generation of probes for

traceable targeted drug/gene delivery [53-55]. CdSe QD-amphipol technology for intracellular delivery and real-time imaging of siRNA into cancer cells with significantly reduced cytotoxicity [56] has been reported. 2-vinyl pyridine functionalized silicon quantum dots [57] and QD-peptide conjugates [58] were also developed for SiRNA transfection and therapeutic imaging. Water soluble and cysteamine capped CdTe QD vectors, conjugated with plasmid DNA via electrostatic interaction, have been reported. The formation of QD-DNA complexes allows controllable release of DNA and gene expression in HEK293 cells in the visible mode [59].

QD-decorated plasmid DNA has been utilized for long-term intracellular and intranuclear tracking of CHO-K1 cells by conjugating plasmid DNA to phospholipid/polyethylene oxide-encapsulated CdSe/ZnS QDs via PNA–SPDP linker [60]. The problem associated with CdSe QDs was addressed by developing low cytotoxic ZnO QD based non-viral vectors with dual functions of delivering plasmid DNA and labeling cells through surface modification with PDMAEMA (poly(2-(dimethylamino)ethylmethylacrylate)). Herein, three PDMAEMA-ZnO QDs, namely QD-1, QD-2, and QD-3, respectively, were prepared on the basis of increasing MAA (methacrylate) content. The PDMAEMA–modified ZnO quantum dots can be used as nonviral vectors for gene delivery due to the ability of the external positive PDMAEMA and internal negative polymethacrylate to condense DNA (**Fig. 6**).

Fig. 6. ZnO QD/pDNA complex formation [61].

Among all the three QDs, QD-3 fails to protect DNA effectively (exhibiting lower transfection efficiency) due to weak electrostatic interaction between ZnO QDs and DNA resulting from higher content of the MAA unit in the copolymer, which profoundly weakens the ability of tertiary amines in PDMAEMA to condense DNA due to the reduction of net positive charge density [61]. It is anticipated that the polycation capped ZnO QDs hold great potential as a new type of transgenic vector, as they could mediate an efficient transfer of plasmid DNA into CoS-7 cells with much lower toxicity, and allowed real imaging of gene transfection in live cells.

Chitosan encapsulated ZnO quantum dots: A smart drug delivery nanocarrier

Semiconductor quantum dots, known as tiny light-emitting nanoparticles, are a new class of fluorescent labels for drug delivery systems and for imaging applications. In many cases, the malignancy of tumors is detected only at advanced stages when chemotherapeutic drugs become increasingly toxic to healthy cells. To improve this condition, both targeted drug delivery [62] and early detection of cancer cells need to be extensively investigated [63]. Tumor targeting drug delivery systems generally combine a tumor recognition moiety such as folate receptors that are overexpressed on tumor cells with a drug loaded vesicle [64-67].

Chitosan, a natural polymer of *N*-acetyl glucosamine and D-glucosamine, with one amino group and two hydroxyl groups, is attractive for the encapsulation of quantum dots. Chitosan encapsulated nanoparticles are good drug carriers and have attracted increasing attention for their wide applications in, for example, loading protein drugs, gene drugs, and anticancer chemical drugs because chitosan enables properties such as (a) chelation of metal ions, water solubility and ease of processing [68-70], (b) easier ligand attachment for targeted drug delivery through the reactive amine group, (c) bio-compatibility and non-cytotoxicity [71], (d) strong electrostatic interaction with negative charged biomolecules and quantum dots [72], and (e) the solubility of chitosan in mild acid (endosomal pH 5.3) and insolubility in physiological pH (7.4) prevents the untimely release of the encapsulated drug before the target site is reached.
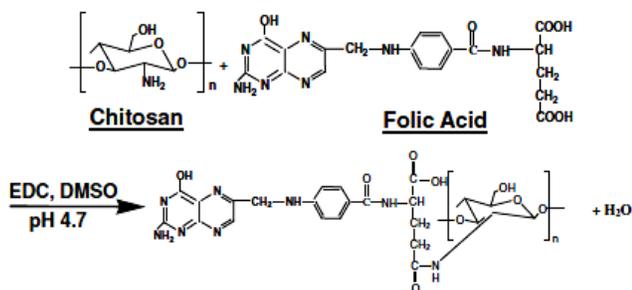
Numerous quantum dots-based drug delivery systems have been explored, because the long-term fluorescence stability of the QDs is very attractive for visualization of drug distribution *in vivo*. CdSe core quantum dots are currently being researched and have shown long term fluorescence stability [72]. The unique optical properties of CdSe/ZnS luminescent QDs have been utilized to examine tumor cell selection internalization for anticancer drugs [73]. Although the use of CdSe core quantum dots for *in vivo* visualization was demonstrated, CdSe/ZnS QDs were found to be cytotoxic upon oxidation and UV exposure, even after the toxic cadmium is encapsulated in a protective shell of ZnS, destroying the QD effect [74]. Thus they are not appropriate as an *in vivo* experimental tool.

In recent years, ZnO quantum dots have been investigated as multifunctional smart drug delivery nanocarriers due to their low toxicity. The "ZnO-chitosan-folate" system can be used as a nanocarrier for delivery of doxorubicin (DOX), an antineoplastic agent used in tumor treatments, through physical and chemical interactions [75]. The scheme for the synthesis of biofunctionalized chitosan and subsequent encapsulation of QDs to obtain ZnO-QD–chitosan–folate carriers is given in **Fig. 7**, and it also shows the incorporation of the anti-cancer drug (doxorubicin hydrochloride, DOX) as Step 3.

The rate of drug release from the nanocarrier depends on several factors, including pH, particle size, surface properties, degradation rate, interaction force of drug binding to the surface and rates of hydration and dehydration of the polymers [76, 77]. Conventionally, drugs are loaded into the nanoparticle via weak interactions, *e.g.* physical adsorption, electrostatic interaction, and p–p stacking and, as a result, the release of drug is achieved by breaking of these interactions. The presence of folic acid in the ZnO-QD-chitosan-folate carrier weakens the electrostatic interaction between DOX and ZnO QDs accounting for the release of DOX.

In another work, ZnO QDs have also been evaluated as a platform for targeted and pH responsive intracellular delivery of DOX [78]. Herein, acidic conditions cause rapid dissolution of ZnO QDs by disfavoring the reaction between Zn^{2+} and DOX, resulting in the release of DOX molecules to the cytosols, killing the cancer cells. Hence, this approach provides a valuable ZnO QDs-based nanovector that can simultaneously realize targeting, diagnosis, and therapy of cancer cells. Recent work [73, 78] points towards the application of water dispersed ZnO quantum dots with long term fluorescence stability in the design of new drug release carriers.

Fig. 7. Preparation of folate conjugated chitosan (1), encapsulation of ZnO QDs with folate-conjugated chitosan (2) and the drug-loading step (3), where biofunctionalized chitosan loaded with drug is used to encapsulate QDs [74].

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Gd doped ZnO quantum dots: Excellent magnetic resonance and fluorescence imaging (MRI-FI) nanoprobes

Magnetic resonance imaging (MRI), one of the most important noninvasive imaging techniques, has been widely used in radiology to visualize detailed internal structures. This technique has certain advantages, such as deep penetration into tissue, providing anatomical details and high quality three dimensional images of soft tissue in a non-invasive monitoring manner [79, 80], but lower sensitivity and its inability to resolve objects larger than a few micrometers in size makes this technique less beneficial for bio-medical application. On the contrary, the fluorescence imaging (FI) technique promises higher sensitivity and the potential for real-time imaging, but the major drawback of this technique is the limited spatial resolution, making it difficult to translate two-dimensional information to the three-dimensional surgical field [81, 82]. Therefore, it was suggested that the limitations associated with both the techniques can be effectively overcome by integrating magnetic resonance and optical imaging functionalities into a single nanostructure [83]. Several different strategies have been directed to developing MRI-FI nanoprobes due to their prominent advantages for medical diagnosis, such as paramagnetic ions doped quantum dots [84-86] and silica encapsulated quantum dots [87]. However, encapsulation by silica shell may lead to difficulty in single probe detection, as this strategy increases the particle size, which is not suitable for labeling functional subcellular proteins [88, 89].

Table 2. Band gap variation with different size of Gd doped ZnO quantum dots [89].

ZnO QDs with different X values	Size[a] (nm)	Size[b] (nm)	Band gap[c] (eV)
0	6	5.7	3.42
0.02	5.18	5.11	3.435
0.04	5.02	4.96	3.44
0.05	4.39	4.36	3.47
0.08	4.14	3.9	3.49
0.10	3.95	3.8	3.50
0.12	3.76	3.56	3.52
0.30	3.55	3.27	3.55

[a]measured by XRD, [b]determined using Meulenkamp's empirical formula, [c]measured by UV-Vis spectra.

A simple and versatile method was used to develop excellent MRI-FI nanoprobes by doping Gd ion in ZnO QDs [90]. These nanoprobes are T_1 positive contrast agents that provide better reliability for clinical diagnosis by

fulfilling modern medical criteria such as (a) high relaxivity and quantum yield, (b) small size, (c) simple cost-effectiveness, (d) low toxicity, and (e) chemical stability in air for optimal therapy. **Table 2** demonstrates the variation of band gap with different X (actual molar ratio of Gd/Zn) values. On decreasing the X value, blue shift of fluorescence spectra of ZnO QDs is observed owing to the quantum confinement.

The nanoprobes, with exceptionally small size and enhanced fluorescence resulting from Gd doping, are ideally suitable for biological and medical fields. They can successfully label HeLa cells in a short time and show no evidence of toxicity on cell growth, even at concentrations up to 1 mM, especially in comparison with the traditional PEGylated CdSe/ZnS or CdSe/CdS QDs, suggesting Gd-doped nanoprobes would find a broad range of applications in the biomedical field by functionalizing these nanoprobes with target ligands.

Bio-mimetic ZnO quantum dots: Novel sensors for allergens and antigens

ZnO quantum dots (QDs) have been increasingly utilized as labeling probes because of their unique properties, such as high aspect ratio, substantial optical and electronic signal amplification and unique coding capabilities [**91-93**]. The isoelectric point (IEP) of ZnO is as high as about 9.5, which is suitable for immobilization of biomolecules with low IEP through electrostatic attraction [**94**] and also ZnO's transparency under visible light and its high environmental and electrical stability make it suitable for biosensing [**95**]. Carbohydrate antigen 19-9 (CA 19-9), a preferred label for pancreatic cancer, is highly lethiferous sarcomata and difficult to be diagnosed early. Although several methods, such as electric field-driven assay [**96**], immobilized horseradish peroxidase assay [**97**], and chemiluminescent multiplex assay [**98**] have been used for the detection of CA 19-9, none has so far been free from certain disadvantages. Recently, sandwich-type immunoassays to detect the carbohydrate antigen (CA 19-9) have been developed [**99**].

A sandwich type structure is formed by the conjugation of ZnO quantum dots with the antibody for CA 19-9 through immunoreactions of CA 19-9 antibodies and antigen. The ZnO QDs linked to the substrate were dissolved in acidic media, and the solution containing Zn^{2+} was accumulated at the electrode and analyzed by square wave stripping voltammetry (SWSV). The resulting immunosensor exhibited high sensitivity and selectivity in detection of antigens due to stable immobilization based on the high IEP of ZnO. The biomimetic nature and high isoelectric point of ZnO QD is also favorable for the construction of a QD-biomolecule assembly for development of biological quantum probes. A concanavalin A (Con A) based sensor for direct electrochemical detection of allergy chicken (CHOM) based on ZnO QDs and a CHOM bioconjugate has been presented [**100**]. The CHOM molecules accumulated onto ZnO QDs through electrostatic interaction between the positively charged QDs surface (IEP = 9.5) and the negatively charged protein (IEP = 3.9). The high surface-to-volume ratio of ZnO QDs led to signal amplification for highly sensitive detection of allergens.

ZnO/Au nanocomposite: A fluorescent sensing platform for DNA detection

Detection of biomolecules such as peptides, proteins and DNA has been a topic of significant interest due to their wide application in gene therapy, drug discovery, and biomedical studies. For high sensitivity and selectivity of gene detection, several methods have been developed in the past few years [**101-103**]. Most of the methods are based on target hybridization with fluorescence but strong background autofluorescence and low signal sensitivity limits the detection of biomolecules using fluorescence measurements [**104**]. Compared to fluorescence, a detection method based on the Raman signal [**105**] has the advantage that Raman bands are 10-100 times narrower and the Raman responses are less susceptible to photobleaching, enabling the possibility of extended signal averaging to lower detection limits. ZnO quantum dots have characteristic resonance multiple-phonon Raman lines, with an excitation wavelength of 325 nm, which can be used as a characteristic fingerprint signal. Hence, they are very promising materials for analyzing various biological macromolecules such as proteins and DNA. Thiol-oligonucleotide-modified ZnO/Au nanocomposite probes have been developed for the detection of specific DNA target sequence [**106**] by monitoring the resonant Raman signal of the ZnO/Au nanocomposites on the Au film. A 30 sequence oligonucleotide DNA2 was used as a model target strand, and half of the target sequences were unknown. Polymerase chain reaction (PCR) was employed to amplify the other half of the target, the complement part of DNA1. The 15- nucleotide capture strands (DNA1) were first bound onto a thin gold film via the Au-S covalent bond. Similarly, the ZnO/Au nanocomposites were easily conjugated with the oligonucleotides (DNA3) functionalized with thiols. The ZnO/Au nanocomposites modified with the thiol oligonucleotide (DNA3) probes were then subsequently hybridized with the overhanging portions of the 15-nucleotide target sequence of DNA2. Therefore, the resonant Raman signals from the ZnO/Au-DNA3 probes can be used to accurately detect the presence of the specific target oligonucleotide strands (DNA2).

This method is ultrasensitive, with an effective detection limit of about 1 fM in DNA concentration. In the light of this fact, this method may be useful in diagnosing genetic diseases and drug discovery.

Acetate and nitrate bound ZnO quantum dots: A promising antimicrobial agent
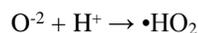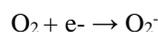
Antibiotic resistance has become a global public health problem; thus it is important to develop new antibacterial agents. The nanostructured materials have been reported to exhibit antibacterial activity against various microorganisms [**107-110**]. Several metal oxide NPs such as TiO_2, MgO and ZnO have been reported to possess significant antibacterial activity and are found to be superior in terms of safety, durability and heat resistance when compared with conventional organic antibacterial agents [**110, 111**].

An increased antimicrobial effect of ZnO nanoparticles was observed against the food related

bacteria *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas fluorescens* **[112]**. There are also other studies confirming the strong antimicrobial activity of ZnO nanoparticles. For example, treatment with ZnO formulation could lyse the food-borne bacteria *Salmonella typhimurium* and *Staphylococcus aureus* **[113]** and also ZnO nanoparticles (12 nm) were found to inhibit the growth of *Escherichia coli* by disintegrating the cell membrane and increasing the membrane permeability **[114]**. Thus, the above findings prove ZnO nanoparticles to be advantageous even in *in vivo* administration, as they contain the mineral elements essential for human beings and can find applications in food systems, inhibiting the growth of pathogenic bacteria.

Several mechanisms have been proposed to explain the antibacterial activity of ZnO nanoparticles. The production of H_2O_2 from the surface of ZnO is considered as an effective means for the inhibition of bacterial growth **[115, 116]**. Another possible mechanism is the release of Zn^{2+} ions, resulting in the damage of the bacterial membrane and direct cellular internalization of ZnO nanoparticles (NPs) **[119]**. A recent study suggested the generation of reactive oxygen species (ROS) by ZnO NPs may lead to oxidative stress and lipid peroxidation, and as a result NPs get internalized resulting in oxidative DNA damage **[120]**.

The ZnO QDs have high concentration of surface defects (loosely bound acetate or nitrate species), which act as trap centers for the conduction electrons **[117]**. Joshi *et al.* studied the role of surface bound anionic species *i.e.* acetate and nitrates, which are conjugate bases of a weak and strong acid, respectively, on ZnO QDs for antibacterial activity against *E. coli* bacteria **[118]**. Under light condition, activation in antibacterial activity of ZnO-Nt (nitrate) QDs and ZnO-Ac (acetate) QDs is attributed to the photoexcitation mechanism where ZnO, being a wide band gap semiconductor, acts as a photocatalyst producing sufficient ROS resulting in the activation of bacterial growth inhibition.

$O_2 + e- \rightarrow O_2^-$

$O^{-2} + H^+ \rightarrow \bullet HO_2$

$\bullet HO_2 + e- + H^+ \rightarrow H_2O_2$

$OH^- + H^+ \rightarrow \bullet OH$

$\bullet OH + \bullet OH \rightarrow H_2O_2$

Under dark condition, ZnO-Ac QDs having acetate defects, show excellent inhibition activity, whereas the presence of the nitrate anion gives very poor inhibition activity. This can be explained as acetate, the conjugate base of a weak acid, attacks the organic molecules and/or functional groups present at the bacterial cell membrane, resulting in the release of the trapped electron at the surface of ZnO, with subsequent reduction of the O_2 present in the surroundings. The formed O^{2-} generates sufficient ROS, causing lipid peroxidation of the cell wall, which further leads to an increase in the membrane's permeability and cellular internalization of ZnO QDs **[119, 121]**. On the contrary, nitrate, being a weak base, has weak reactivity and the conduction band electron of ZnO remains trapped on its surface by the nitrate anion species.

Acetate bound ZnO QDs show significant antibacterial activity, not only in the presence of light, but also in the dark, revealing an important role of surface defects or surface adsorbed anionic species. Thus, surface modifications of ZnO QDs with an appropriate anionic species may lead to the design of more specific bactericidal QDs and the use of ZnO is of great advantage, as it contains a mineral element essential to humans.

Conclusion

In recent years, ZnO quantum dots have attracted attention as very promising candidates for optoelectronic, electronic, and biological applications. Low toxicity, low cost, and biocompatibility makes them excellent candidates for *in vivo* bio-imaging, gene/drug delivery and cancer detection. ZnO quantum dots have also promised significant breakthrough in search for antibacterial agents, and in detection of important antigens and allergens due to their high isoelectric points. This review has highlighted recent advancements in the surface modification of ZnO QDs for bio-applications. Surface modified ZnO quantum dots open up new avenues in the field of biology, as these have good fluorescence property, high quantum yield and chemical stability in aqueous solutions as well.

Future perspectives

QDs have revolutionized the field of medicine. ZnO quantum dots have been gaining attention as promising alternatives to the more widely studied but toxic cadmium based quantum dots. Still, there is an urgent need to study the effect of different surface modifiers on ZnO quantum dots for increased quantum efficiency and better fluorescence stability, which will enable their applications as more sensitive, qualitative and quantitative tools for imaging, diagnosis and better targeting such as drug delivery. The forthcoming years would see their potential applications in different fields such as molecular probes against various biological markers such as free antigens, cell surface markers/antigens, bacteria, viruses and tissues.

Abbreviations

CMCD Carboxymethyl β–cyclodextrin, **FI** Fluorescence imaging, **FRET** fluorescence resonance energy transfer, **IEP** Isoelectric point, **MRI** Magnetic resonance imaging, **MAA** Mercaptoacetic acid, **NPs** Nanoaparticles, **PL QY** Photoluminescence quantum yield, **QDs** Quantum dots, **ROS** Reactive oxygen species.

Reference

1. Rossetti, R.; Nakahara, S.; Brus, L. E. *J. Chem. Phys.* **1983**, *79*, 1086.
   DOI: 10.1063/1.445834
2. Ekimov, A. I.; Efros, Al. L.; Onushchenko, A. A. *Solid State Commun*. **1985**, *56*, 921.
   DOI: 10.1016/S0038-1098(85)80025-9
3. Bruchez, M. Jr.; Moronne, M.; Gin, P.; Weiss, S.; Alivisatos, A. P. *Science*. **1998**, *281*, 2013.

DOI: 10.1126/science.281.5385.2013
Tiwari, A.; Kobayashi, H. (Eds.), *In Responsive Materials and Methods: State-of-the-Art Stimuli-Responsive Materials and Their Applications*, WILEY-Scrivener Publishing LLC, USA, **2013.**

4. Chan, W. C. W.; Nie, S. *Science.* **1998**, *281,* 2016.
    DOI: 10.1126/science.281.5385.2016
    Tiwari, A.; Tiwari, A. (Eds.), *In Nanomaterials in Drug Delivery, Imaging, and Tissue Engineering*, WILEY-Scrivener Publishing LLC, USA, **2013.**

5. Drbohlavova, J.; Adam, V.; Kirzek, R.; Hubalek, J. *Int. J. Mol. Sci.* **2009**, *10,* 656.
    DOI: 10.3390/ijms10020656

6. Aldeek, F.; Balan, L.; Lambert, J.; Schneider, R. *Nanotechnology.* **2008**, *19,* 475401.
    DOI: 10.1088/0957-4484/19/47/475401

7. Law, W. -C.; Yong, K. -T.; Roy, I.; Ding, H.; Hu, R.; Zhao, W.; Prasad, P. N. *Small*. **2009**, *5,* 1302.
    DOI: 10.1002/smll.200801555

8. Dumas, E.; Gao, C.; Suffern, D.; Bradforth, S. E.; Dimitrijevic, N. M.; Nadeau, J. L. *Environ. Sci. Technol.* **2010**, *44,* 1464.
    DOI: 10.1021/es902898d

9. Schneider, R.; Wolpert, C.; Guilloteau, H.; Balan, L.; Lambert, J.; Merlin, C. *Nanotechnology*. **2009**, *20,* 225101.
    DOI: 10.1088/0957-4484/20/22/225101

10. Joo, J.; Kwon, S. G.; Yu, J. H.; Hyeon, T. *Adv. Mater*. **2005**, *17,* 1873.
    DOI: 10.1002/adma.200402109

11. Kohls, M.; Schmidt, T.; Katschorek, H.; Spanhel, L.; Müller, G.; Mais, N.; Wolf, A.; Forchel, A. *Adv. Mater*. **1999**, *11,* 288.
    DOI: 10.1002/(SICI)1521-4095(199903)11:4<288

12. Qu, F.; Santos, D. R. Jr.; Dantas, N. O.; Monte, A. F. G.; Morais, P. C. *Physica E-Low-Dimensional Systems and Nanostructures*. **2004**, *23,* 410.
    DOI: 10.1016/j.physe.2003.12.137

13. Tong, Y. H.; Liu, Y. C.; Lu, S. X.; Dong, L.; Chen, S. J.; Xiao, Z. Y. *J. Sol-Gel. Sci. Techn*. **2004**, *30,* 157.
    DOI: 10.1023/B:JSST.0000039500.48283.5a

14. Lin, K. -F.; Cheng, H. -M.; Hsu, H. -C.; Lin, L. -J.; Hsieh, W. -F. *Chem. Phys. Lett*. **2005**, *409,* 208.
    DOI: 10.1016/j.cplett.2005.05.027
    Chey, C.O.; Patra, H.K.; Tengdelius, M.; Golabi, M.; Parlak, O.; Imani, R.; Elhag, S.A.; Yandi, W.; Tiwari, A. *Adv. Mat. Lett.* **2013**, *4,* 591.

15. Millers, D.; Grigorjeva, L.; Łojkowski, W.; Strachowski, T. *Radiat. Meas.* **2004**, *38,* 589.
    DOI: 10.1016/j.radmeas.2004.05.001
    Tiwari, A.; Ramalingam, M.; Kobayashi, H.; Turner, A.P.F. *In Biomedical materials and diagnostic devices*, WILEY-Scrivener Publishing LLC, USA, **2012.**

16. Gupta, K. C.; Jabrail, F. H. *Carbohyd. Polym.* **2006**, *66,* 43.
    DOI: 10.1016/j.carbpol.2006.02.019

17. Kumar, S.; Gupta, V.; Sreenivas, K. *Nanotechnology* **2005**, *16,* 1167.
    DOI: 10.1088/0957-4484/16/8/031
    Shukla, S.K.; Vamakshi, Minakshi, Bharadavaja, A.; Shekhar, A.; Tiwari, A. *Adv. Mat. Lett.* **2012**, *3,* 421.

18. Tian, Z. R.; Voigt, J. A.; Liu, J.; Mckenzie, B.; Mcdermott, M. J.; Rodriguez, M. A.; Konishi, H.; Xu, H. *Nat. Mater*. **2003**, *2,* 821.
    DOI: 10.1038/nmat1014

19. Wang, Z. L. *J. Phys.: Condens. Matter.* **2004**, *16,* R829.
    DOI: 10.1088/0953-8984/16/25/R01

20. Frasco, M. F.; Chaniotakis, N. *Sensors*. **2009**, *9,* 7266.
    DOI: 10.3390/s90907266

21. Zhang, Y.; Yin, L.; Wang, C.; Lun, N.; Qi, Y.; Xiang, D. *J. Phys. Chem. C.* **2010**, *114,* 9651.
    DOI: 10.1021/jp101324a

22. Xiong, H. -M. *J. Mater. Chem.* **2010**, *20,* 4251.
    DOI: 10.1039/B918413A

23. Lin, K. -F.; Cheng, H. -M.; Hsu, H. -C.; Lin, L. -J.; Hsieh, W. -F. *Chem. Phys. Lett.* **2005**, *409,* 208.
    DOI: 10.1016/j.cplett.2005.05.027

24. Ajimsha, R. S.; Anoop, G.; Aravind, A.; Jayaraj, M. K. *Electrochem. Solid-State Lett.* **2008**, *11,* K14.
    DOI: 10.1149/1.2820767

25. Zhuang, J.; Liu, M.; Liu, H. *Sci. China Ser B-Chem.* **2009**, *52,* 2125.
    DOI: 10.1007/s11426-009-0198-5

26. Tiwari, A.; Mishra, A. K.; Kobayashi, H.; Turner, A. P. F. *Intelligent Nanomaterials*, WILEY-Scrivener Publishing LLC, USA, **2012**, pp. 23-24.
    ISBN: 978-04-709387-99

27. Xiong, H. -M.; Wang, Z. -D.; Xia, Y. -Y. *Adv. Mater.* **2006**, *18,* 748.
    DOI: 10.1002/adma.200501899

28. Xiong, H. -M.; Xie, D. -P.; Guan, X. -Y.; Tan, Y. -J.; Xia, Y. -Y. *J. Mater. Chem.* **2007**, *17,* 2490.
    DOI: 10.1039/B700176B

29. Xiong, H. -M.; Xu, Y.; Ren, Q. -G.; Xia, Y. -Y. *J. Am. Chem. Soc.* **2008**, *130,* 7522.
    DOI: 10.1021/ja800999u

30. Zhang, P.; Liu, W. *Biomaterials*. **2010**, *31,* 3087.
    DOI: 10.1016/j.biomaterials.2010.01.007

31. Yan, E.; Wang, C.; Wang, S.; Sun, L.; Wang, Y.; Fan, L.; Zhang, D. *Mater. Sci. Eng. B*. **2011**, *176,* 458.
    DOI: 10.1016/j.mseb.2011.01.005

32. Shukla, S. K.; Deshpande, S. R.; Shukla, S. K.; Tiwari, A. *Talanta* **2012**, *99,* 283.
    DOI: 10.1016/j.talanta.2012.05.052
    Shukla, S.K.; Tiwari, A.; Parashar, G.K.; Mishra, A.P.; Dubey, G.C. *Talanta*, **2009**, *80,* 565.

33. Bruce, I. J.; Sen, T. *Langmuir*. **2005**, *21,* 7029.
    DOI: 10.1021/la050553t

34. Soares, J. W.; Whitten, J. E.; Oblas, D. W.; Steeves, D. M. *Langmuir*. **2008**, *24,* 371.
    DOI: 10.1021/la702834w

35. Jana, N. R.; Yu, H. -H.; Ali, E. M.; Zheng, Y.; Ying, J. Y. *Chem. Commun.* **2007**, *1406.*
    DOI: 10.1039/B613043G

36. Wu, Y. L.; Lim, C. S.; Fu, S.; Tok, A. I. K.; Lau, H. M.; Boey, F. Y. C.; Zheng, X. T. *Nanotechnology*. **2007**, *18,* 215604.
    DOI: 10.1088/0957-4484/18/21/215604

37. Moussodia, R. -O.; Balan, L.; Merlin, C.; Mustin, C.; Schneider, R. *J. Mater. Chem.* **2010**, *20,* 1147.
    DOI: 10.1039/B917629B

38. Rakshit, S.; Vasudevan, S. *ACS Nano*. **2008**, *2,* 1473.
    DOI: 10.1021/nn800152a

39. Liu, J.; Mendoza, S.; Román, E.; Lynn, M. J.; Xu, R.; Kaifer, A. E. *J. Am. Chem. Soc.* **1999**, *121,* 4304.
    DOI: 10.1021/ja990330n

40. Kachynski, A. V.; Kuzmin, A. N.; Nyk, M.; Roy, I.; Prasad, P. N. *J. Phys. Chem. C.* **2008**, *112,* 10721.
    DOI: 10.1021/jp801684j

41. Cao, Y. C. *Science*. **2011**, *332,* 48.
    DOI: 10.1126/science.1203702

42. Norris, D. J.; Efros, A. L.; Erwin, S. C. *Science*. **2008**, *319,* 1776.
    DOI: 10.1126/science.1143802

43. Wang, F.; Liu, X. *Chem. Soc. Rev*. **2009**, *38,* 976.
    DOI: 10.1039/B809132N

44. Ntwaeaborwa, O. M.; Holloway, P. H. *Nanotechnology*. **2005**, *16,* 865.
    DOI: 10.1088/0957-4484/16/6/042

45. Yu, Y.; Chen, D.; Huang, P.; Lin, H.; Wang, Y. *Ceram. Int.* **2010**, *36,* 1091.
    DOI: 10.1016/j.ceramint.2009.12.007

46. Gu, F.; Wang, S. F.; Lü, M. K.; Zhou, G. J.; Xu, D.; Yuan, D. R. *Langmuir*. **2004**, *20,* 3528.
    DOI: 10.1021/la049874f

47. Karthikeyan, B.; Sandeep, C. S. S.; Pandiyarajan, T.; Venkatesan, P.; Philip, R. *Appl. Phys. A: Mater. Sci. Process.* **2011**, *102,* 115.
    DOI: 10.1007/s00339-010-6014-4

48. Jia, T.; Wang, W.; Long, F.; Fu, Z.; Wang, H.; Zhang, Q. *Mater. Sci. Eng. B.* **2009**, *162,* 179.
    DOI: 10.1016/j.mseb.2009.04.004

49. Liu, Y.; Xu, C.; Yang, Q. *J. Appl. Phys.* **2009**, *105,* 084701.
    DOI: 10.1063/1.3088881

50. Liu, Y.; Ai, K.; Yuan, Q.; Lu, L. *Biomaterials*. **2011**, *32,* 1185.
    DOI: 10.1016/j.biomaterials.2010.10.022

51. Dutta, R. K.; Sharma, P. K.; Pandey, A. C. *J. Nanopart. Res*. **2010**, *12,* 1211.
    DOI: 10.1007/s11051-009-9801-0

52. Katragadda, C. S.; Choudhury, P. K.; Murthy, P. N. *Indian J. Pharm. Educ. Res.* **2010**, *44,* 109.

53. Al-Jamal, W. T.; Al-Jamal, K. T.; Tian, B.; Cakebread, A.; Halket, J. M.; Kostarelos, K. *Mol. Pharmaceutics*. **2009**, *6,* 520.

DOI: 10.1021/mp800187d

54. Qi, L.; Gao, X. *Expert. Opin. Drug Deliv.* **2008**, *5*, 263.

55. Yezhelyev, M. V.; Qi, L.; O'Regan, R. M.; Nie, S.; Gao, X. *J. Am. Chem. Soc.* **2008**, *130*, 9006.
DOI: 10.1021/ja800086u

56. Qi, L.; Gao, X. *ACS Nano*. **2008**, *2*, 1403.
DOI: 10.1021/nn800280r

57. Klein, S.; Zolk, O.; Fromm, M. F.; Schrödl, F.; Neuhuber, W.; Kryschi, C. *Biochem. Biophys. Res. Commun.* **2009**, *387*, 164.
DOI: 10.1016/j.bbrc.2009.06.144

58. Walther, C.; Meyer, K.; Rennert, R.; Neundorf, I. *Bioconjugate Chem.* **2008**, *19*, 2346.
DOI: 10.1021/bc800172q

59. Li, D.; Li, G. P.; Guo, W. W.; Li, P. C.; Wang, E. K.; Wang, J. *Biomaterials*. **2008**, *29*, 2776.
DOI: 10.1016/j.biomaterials.2008.03.007

60. Srinivasan, C.; Lee, J.; Papadimitrakopoulos, F.; Silbart, L. K.; Zhao, M. H.; Burgess, D. J. *Mol. Ther*. **2006**, *14*, 192.
DOI: 10.1016/j.ymthe.2006.03.010

61. Zhang, P.; Liu, W. *Biomaterials*. **2010**, *31*, 3087.
DOI: 10.1016/j.biomaterials.2010.01.007

62. Moghimi, S. M.; Rajabi-Siahboomi, A. R. *Adv. Drug Deliver. Rev*. **2000**, *41*, 129.
DOI: 10.1016/S0169-409X(99)00060-5

63. Jaracz, S.; Chen, J.; Kuznetsova, L. V.; Ojima, I. *Bioorg. Med. Chem*. **2005**, *13*, 5043.
DOI: 10.1016/j.bmc.2005.04.084

64. Antony, A. C. *Annu. Rev. Nutr.* **1996**, *16*, 501. Folate receptors.
DOI: 10.1146/annurev.nu.16.070196.002441

65. Lu, Y.; Low, P. S. *Adv. Drug Deliver. Rev*. **2002**, *54*, 675.
DOI: 10.1016/S0169-409X(02)00042-X

66. Maziarz, K. M.; Monaco, H. L.; Shen, F.; Ratnam, M. *J. Biol. Chem.* **1999**, *274*, 11086.
DOI: 10.1074/jbc.274.16.11086

67. Weitman, S. D.; Lark, R. H.; Coney, L. R.; Fort, D. W.; Frasca, V.; Zurawski, V. R. Jr.; Kamen, B. A. *Cancer Res*. **1992**, *52*, 3396.

68. Dutta, P. K.; Ravikumar, M. N. V.; Dutta, J. *J. Macromol. Sci., Part C- Polym. Rev.* **2002**, *C42*, 307.
DOI: 10.1081/MC-120006451
Mourya, V.K.; Inamdar, N.N.; Tiwari, A. *Adv. Mat. Lett.* **2010**, *1*, 11.

69. Shukla, S.K.; Mishra A.K.; Arotiba, O.A.; Mamba, B.B. *Int. J. Biol. Macromol.* **2013**, 59, 46.
DOI: 10.1016/j.ijbiomac.2013.04.043
Tiwari, A. *Journal of Inorganic and Organometallic Polymers and Materials*, **2009**, *19*, 361.

70. Hirano, S. *Biotechnol. Annu. Rev*. **1996**, *2*, 237.
DOI: 10.1016/S1387-2656(08)70012-7

71. Gupta, K. C.; Jabrail, F. H. *Carbohyd. Polym*. **2006**, *66*, 43.
DOI: 10.1016/j.carbpol.2006.02.019

72. Jamieson, T.; Bakhshi, R.; Petrova, D.; Pocock, R.; Imani, M.; Seifalian, A. M. *Biomaterials*. **2007**, *28*, 4717.
DOI: 10.1016/j.biomaterials.2007.07.014

73. Weng, K. C.; Noble, C. O.; Papahadjopoulos-Sternberg, B.; Chen, F. F.; Drummond, D. C.; Kirpotin, D. B.; Wang, D.; Hom, Y. K.; Hann, B.; Park, J. W. *Nano Lett*. **2008**, *8*, 2851.
DOI: 10.1021/nl801488u

74. Derfus, A. M.; Chan, W. C. W.; Bhatia, S. N. *Nano Lett.* **2004**, *4*, 11.
DOI: 10.1021/nl0347334

75. Yuan, Q.; Hein, S.; Misra, R. D. K. *Acta Biomater*. **2010**, *6*, 2732.
DOI: 10.1016/j.actbio.2010.01.025

76. Yuan, Q.; Venkatasubramanian, R.; Hein, S.; Misra, R. D. K. *Acta Biomater*. **2008**, *4*, 1024.
DOI: 10.1016/j.actbio.2008.02.002
Ramalingam, M.; Tiwari, A.; Ramakrishna, S.; Kobayashi, H. In Integrated Biomaterials for Biomedical Technology, WILEY-Scrivener Publishing LLC, USA, **2012.**

77. Zhang, J.; Misra, R. D. K. *Acta Biomater*. **2007**, *3*, 838.
DOI: 10.1016/j.actbio.2007.05.011
Tiwari, A.; Prabaharan, M. *Journal of Biomaterials Science, Polymer Edition*, **2010**, *21*, 937.

78. Muhammad, F.; Guo, M.; Guo, Y.; Qi, W.; Qu, F.; Sun, F.; Zhao, H.; Zhu, G. *J. Mater. Chem*. **2011**, *21*, 13406.
DOI: 10.1039/C1JM12119G

79. Lauffer, R. B. *Chem. Rev*. **1987**, *87*, 901.
DOI: 10.1021/cr00081a003

80. Mulder, W. J. M.; Strijkers, G. J.; van Tilborg, G. A. F.; Griffioen, A. W.; Nicolay, K. *NMR Biomed*. **2006**, *19*, 142.
DOI: 10.1002/nbm.1011

81. Bridot, J. -L.; Faure, A. -C.; Laurent, S.; Rivière, C.; Billotey, C.; Hiba, B.; Janier, M.; Josserand, V.; Coll, J. -L.; Elst, L. V.; Muller, R.; Roux, S.; Perriat, P.; Tillement, O. *J. Am. Chem. Soc.* **2007**, *129*, 5076.
DOI: 10.1021/ja068356j
Patra, H.K.; Khaliq, N.U.; Romu, T.; Wiechec, E.; Borga, M.; Turner, A.P.F.; Tiwari, A. Advanced Healthcare Materials, Published Online, **2013**.
DOI: 10.1002/adhm.201300225

82. Tsai, C. -P.; Hung, Y.; Chou, Y. -H.; Huang, D. -M.; Hsiao, J. -K.; Chang, C.; Chen, Y. -C.; Mou, C. -Y. *Small*. **2008**, *4*, 186.
DOI: 10.1002/smll.200700457

83. Lewin, M.; Carlesso, N.; Tung, C. -H.; Tang, X. -W.; Cory, D.; Scadden, D. T.; Weissleder, R. *Nat. Biotechnol*. **2000**, *18*, 410.
DOI: 10.1038/74464

84. Li, I. -F.; Yeh, C. -S. *J. Mater. Chem*. **2010**, *20*, 2079.
DOI: 10.1039/b924089f

85. Santra, S.; Yang, H.; Holloway, P. H.; Stanley, J. T.; Mericle, R. A. *J. Am. Chem. Soc*. **2005**, *127*, 1656.
DOI: 10.1021/ja0464140

86. Wang, S.; Jarrett, B. R.; Kauzlarich, S. M.; Louie, A. Y. *J. Am. Chem. Soc.* **2007**, *129*, 3848.
DOI: 10.1021/ja065996d

87. Ai, K.; Zhang, B.; Lu, L. *Angew. Chem. Int. Ed*. **2009**, *48*, 304.
DOI: 10.1002/anie.200804231

88. Drummond, D. C.; Meyer, O.; Hong, K.; Kirpotin, D. B.; Papahadjopoulos, D. *Pharmacol. Rev.* **1999**, *51*, 691.

89. Medintz, I. L.; Uyeda, H. T.; Goldman, E. R.; Mattoussi, H. *Nat. Mater*. **2005**, *4*, 435.
DOI: 10.1038/nmat1390

90. Liu, Y.; Ai, K.; Yuan, Q.; Lu, L. *Biomaterials*. **2011**, *32*, 1185.
DOI: 10.1016/j.biomaterials.2010.10.022

91. Penn, S. G.; He, L.; Natan, M. *J. Curr. Opin. Chem. Biol*. **2003**, *7*, 609.
DOI: 10.1016/j.cbpa.2003.08.013

92. Rosi, N. L.; Mirkin, C. A. *Chem. Rev*. **2005**, *105*, 1547.
DOI: 10.1021/cr030067f

93. Wang, J. *Small*. **2005**, *1*, 1036.
DOI: 10.1002/smll.200500214

94. Gu, B. X.; Xu, C. X.; Zhu, G. P.; Liu, S. Q.; Chen, L. Y.; Li, X. S. *J. Phys. Chem. B*. **2009**, *113*, 377.
DOI: 10.1021/jp808001c

95. Brayner, R.; Ferrari-lliou, R.; Brivois, N.; Djediat, S.; Benedetti, M. F.; Fiévet, F. *Nano Lett*. **2006**, *6*, 866.
DOI: 10.1021/nl052326h

96. Wu, J.; Yan, Y.; Yan, F.; Ju, H. *Anal. Chem*. **2008**, *80*, 6072.
DOI: 10.1021/ac800905k

97. Dan, D.; Xiaoxing, X.; Shengfu, W.; Aidong, Z. *Talanta.* **2007**, *71*, 1257.
DOI: 10.1016/j.talanta.2006.06.028

98. Fu, Z. F.; Yang, Z.; Tang, J.; Liu, H.; Yan, F.; Ju, H. *Anal. Chem*. **2007**, *79*, 7376.
DOI: 10.1021/ac0711900

99. Gu, B.; Xu, C.; Yang, C.; Li, S.; Wang, M. *Biosens. Bioelectron*. **2011**, *26*, 2720.
DOI: 10.1016/j.bios.2010.09.031

100. Yang, C.; Gu, B.; Xu, C.; Xu, X. *J. Electroanal. Chem.* **2011**, *660*, 97.
DOI: 10.1016/j.jelechem.2011.06.011

101. Hansen, J. A.; Mukhopadhyay, R.; Hansen, J. Ø.; Gothelf, K. V. *J. Am. Chem. Soc.* **2006**, *128*, 3860.
DOI: 10.1021/ja0574116

102. Wang, J.; Xu, D.; Polsky, R. *J. Am. Chem. Soc.* **2002**, *124*, 4208.
DOI: 10.1021/ja0255709

103. Zhao, X.; Tapec-Dytioco, R.; Tan, W. *J. Am. Chem. Soc*. **2003**, *125*, 11474.
DOI: 10.1021/ja0358854

104. So, M. -K.; Xu, C.; Loening, A. M.; Gambhir, S. S.; Rao, J. *Nat. Biotechnol*. **2006**, *24*, 339.
DOI: 10.1038/nbt1188

105. Grubisha, D. S.; Lipert, R. J.; Park, H. -Y.; Driskell, J.; Porter, M. D. *Anal. Chem*. **2003**, *75*, 5936.
DOI: 10.1021/ac034356f

106. Liu, Y.; Zhong, M.; Shan, G.; Li, Y.; Huang, B.; Yang, G. *J. Phys. Chem. B*. **2008**, *112*, 6484.
    DOI: 10.1021/jp710399d

107. Kim, J. S.; Kuk, E.; Yu, K. N.; Kim, J. -H.; Park, S. J.; Lee, H. J.; Kim, S. H.; Park, Y. K.; Park, Y. H.; Hwang, C. -Y.; Kim, Y. -K.; Lee, Y. -S.; Jeong, D. H.; Cho, M. -H. *Nanomed. Nanotechnol*. **2007**, *3*, 95.
    DOI: 10.1016/j.nano.2006.12.001

108. Ruparelia, J. P.; Chatterjee, A. K.; Duttagupta, S. P.; Mukherji, S. *Acta Biomater*. **2008**, *4*, 707.
    DOI: 10.1016/j.actbio.2007.11.006

109. Shrivastava, S.; Bera, T.; Roy, A.; Singh, G.; Ramachandrarao, P.; Dash, D. *Nanotechnol*. **2007**, *18*, 225103.
    DOI: 10.1088/0957-4484/18/22/225103

110. Makhluf, S.; Dror, R.; Nitzan, Y.; Abramovich, Y.; Jelinek, R.; Gedanken, A. *Adv. Funct. Mater*. **2005**, *15*, 1708.
    DOI: 10.1002/adfm.200500029

111. Stoimenov, P. K.; Klinger, R. L.; Marchim, G. L.; Klabunde, K. J. *Langmuir*. **2002**, *18*, 6679.
    DOI: 10.1021/la0202374

112. Jiang, W.; Mashayekhi, H.; Xing, B. *Environ. Pollut.* **2009**, 157, 1619.
    DOI: 10.1016/j.envpol.2008.12.025

113. Liu, Y.; He, L.; Mustapha, A.; Li, H.; Hu, Z. Q.; Lin, M. *J. Appl. Microbiol*. **2009**, *107*, 1193.
    DOI: 10.1111/j.1365-2672.2009.04303.x

114. Jin, T.; Sun, D.; Su, Y.; Zhang, H.; Sue, H. -J. *J. Food Sci.* **2009**, *74*, M-46.
    DOI: 10.1111/j.1750-3841.2008.01013.x

115. Sawai, J.; Kawada, E.; Kanou, F.; Igarashi, H.; Hashimoto, A.; Kokugan, T.; Shimizu, M. *J. Chem. Eng. Jpn*. **1996**, *29*, 627.

116. Yamamoto, O. *Int. J. Inorg. Mater*. **2001**, *3*, 643.
    DOI: 10.1016/S1466-6049(01)00197-0

117. Yadav, H. K.; Sreenivas, K.; Gupta, V.; Singh, S. P.; Katiyar, R. S. *J. Mater. Res*. **2007**, *22*, 2404.
    DOI: 10.1557/jmr.2007.0321

118. Joshi, P.; Chakraborti, S.; Chakrabarti, P.; Haranath, D.; Shanker, V.; Ansari, Z. A.; Singh, S. P.; Gupta, V. **2009**, *9*, 6427.
    DOI: 10.1166/jnn.2009.1584

119. Brayner, R.; Ferrari-lliou, R.; Brivois, N.; Djediat, S.; Benedetti, M. F.; Fiévet, F. *Nano Lett*. **2006**, *6*, 866.
    DOI: 10.1021/nl052326h

120. Lin, W.; Xu, Y.; Huang, C. -C.; Ma, Y.; Shannon, K. B.; Chen, D.-R.; Huang, Y. -W. *J. Nanopart. Res*. **2009**, *11*, 25.
    DOI: 10.1007/s11051-008-9419-7

121. Nel, A.; Xia, T.; Mädler, L.; Li, N. *Science*. **2006**, *311*, 622.
    DOI: 10.1126/science.1114397