Expert Opinion

Advances and challenges of nanotechnology-based drug delivery systems

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The ability to deliver highly efficient therapeutic compounds specifically to diseased sites is crucial for effectively treating all human illnesses. Unfortunately, conventional therapeutic strategies require unnecessarily high systemic administration due to non-specific biodistribution and rapid metabolism of free drug molecules prior to reaching their targeted sites. Using the tools of nanotechnology, drug delivery systems within the nanometer size regime can be developed to alter both pharmacological and therapeutic effects of drug molecules. Due to their small size, these novel DDS offer superior advantages, such as altered pharmacokinetic behaviour and improved payload, over traditional large-scale systems. In addition, the relative ease in modifying their surface chemistry permits the attachment of targeting and therapeutic molecules for specific therapeutic applications. Finally, complex nanostructures can be assembled using different building blocks with multiple functionalities ranging from targeting, detecting, imaging and therapeutic capabilities.

Keywords: buckyballs, carbon nanotubes, drug delivery system, liposomes, nanostructures, quantum dots

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1. Introduction

The many advances in medicine have lead to lengthened life expectancies and significant improvements in our general health conditions. Notably, research in pharmaceutical sciences has played a crucial role in this progress. From ancient Egyptian, Indian and Chinese herbal remedies to state-of-the-art monoclonal antibody-based therapeutics, both synthetic and naturally-occurring chemical or biological compounds have helped mankind fight and defend against diseases for many generations [1,2]. Although a significant portion of pharmacological compounds has been approved by the FDA for clinical applications, their method of administration remains essentially unchanged, in which drug molecules in their native form are administered through various routes including oral, topical or intravenous injections. Recently, the emergence of biotechnology research has generated great interest in developing novel drug delivery systems (DDSs) to improve both the pharmacological and therapeutic properties of parenterally administered drugs [3,4]. In particular, advances in nanotechnology have produced an array of nanoscaled polymeric, liposomal and inorganic materials as potential drug carriers (**Figure 1**) [5-7]. Unlike large-scale DDSs, nanomaterial-based DDSs (nano-DDSs) offer easier penetration through certain regions of the body due to their small size [8], simplicity in surface modification [8] and, in some cases, multifunctional capabilities for simultaneous therapeutic and imaging applications [9]. In this review, the authors highlight several prominent nanomaterials and their potential uses in DDSs, as well as

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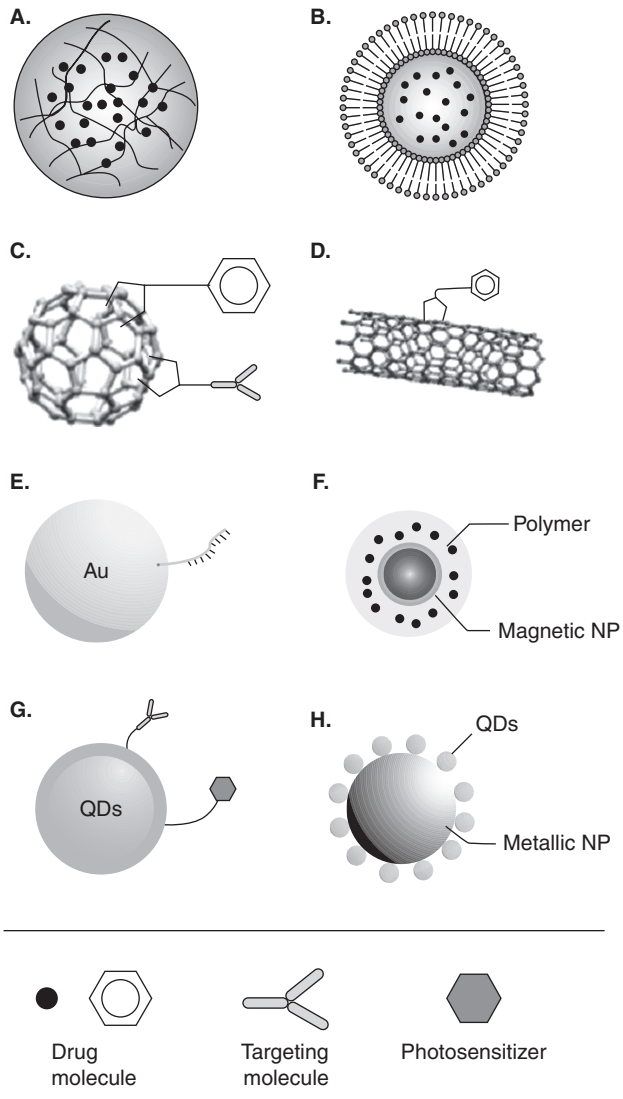

Figure 1. Various nanomaterial-based drug delivery platforms. A. Polymeric nanoparticles/micelles **B.** Liposome **C.** Buckyball **D.** Carbon nanotube **E.** Colloidal gold nanoparticle **F.** Magnetic nanoparticle **G.** Quantum dots **H.** Multifunctional nanoparticle with metallic nanoparticle core (metallic nanoparticle) and semiconductor quantum dots surrounding the shell. Drug molecules can be attached to these carrier systems through encapsulation, mixing, covalent conjugation or electrostatic and affinity interactions.

CNT: Carbon nanotube; NP: Nanoparticle; QD: Quantum dot.

important challenges to overcome before their clinical implementation can be realized.

2. Why nano-drug delivery systems?

One of the most appealing properties of nano-DDSs is their size. Very similar to biological entities, such as a virus, typical nano-DDSs have at least one dimension that is within the size range of 1 – 100 nm [10]. Their small physical dimensions enable them to penetrate through biological and physiological barriers that are normally impermeable for larger particulate structures (**Figure 2**) [8]. In addition, nano-DDS surfaces can easily be modified using conventional chemical techniques to alter and tune pharmacokinetic or pharmacodynamic properties [11]. For example, polyethyleneglycol (PEG) linkage onto nanocarrier surfaces dramatically increases their circulation time within the body, thus reducing non-specific uptake and harboring by the reticuloendothelial system [12]. The flexible surface chemistry of nano-DDSs also allows the ability to conjugate targeting ligands. Biological moieties such as antibodies, peptides and oligonucleotide sequences can be attached to their surfaces to target drugs to specific diseased sites [13]. Within the human body, targeted nano-DDS delivery can increase drug payload while significantly reducing the risk of adverse systemic side effects, leading to better patient compliance and enhanced therapeutic efficacy [14]. Furthermore, the ability to alter the size, shape and composition of nanostructures during synthesis has significant implications for present drug delivery strategies. Both *in vitro* and *in vivo* studies have demonstrated that nanostructures of different sizes and shapes dictate their ability to enter cells and tissues [15,16]. For example, the cellular uptake of colloidal nanoparticles is most efficient within the 50-nm size range due to energetic limitations involved in membrane remodeling [17]. In addition, elongated nanostructures, such as wires and rods have been shown to possess a longer circulation time *in vivo*, resulting in reduced metabolic clearance [16]. Finally, the ability to construct complex nano-DDSs with multifunctional capabilities could lead to the development of a universal platform that can simultaneously detect, image and deliver therapeutic compounds. Mulder *et al.* used semiconductor nanocrystals (quantum dots; Qdots) conjugated to paramagnetic lipids for multi-modal combined fluorescence and MRI [18]. Conjugating drug molecules to these heterogeneous nanoparticle surfaces can provide additional functionalities. Potentially, using a single nano-DDS, a patient could be diagnosed with a disease (such as cancer) using an enhanced MRI imaging, while fluorescence emission from Qdots could help physicians perform real-time tumor resection using optical guidance with encapsulated drug molecules providing post-surgical adjuvant therapy. In the following sections, the authors aim to discuss some of the most-studied nano-DDSs. Although classical soft matter nanomaterials including lipid and polymeric nanoparticles have been around for decades, with many already FDA approved for clinical applications, a new generation of nanostructures such as buckyball, carbon nanotube, metallic and semiconductor nanomaterials are also beginning to emerge as possible candidates for nano-DDSs [11,19-22]. Furthermore, hybrid nanostructures comprised of different nanomaterials through self-assembly add new dimensions to traditional drug delivery, as they offer multi-modal capabilities [23]. The advances in nano-DDSs are intriguing, yet many challenges still remain. The elucidation

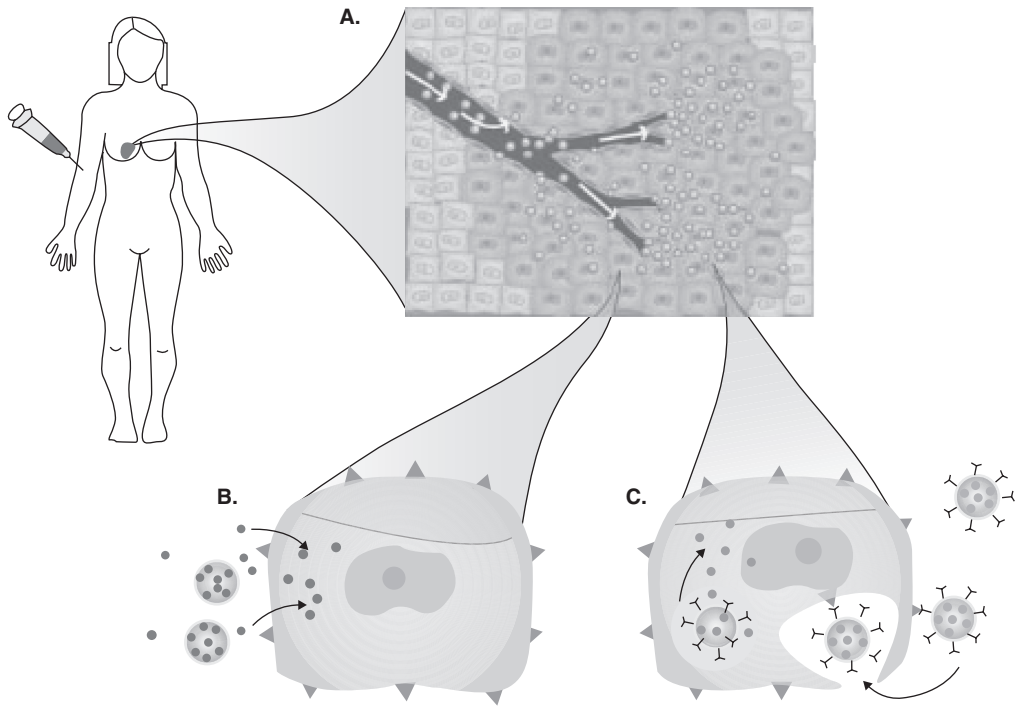

Figure 2. Nano-drug delivery systems *in vivo*. A. Intravenously administered nano-drug delivery systems flow within the bloodstream. **B.** Nano-drug delivery system passive targeting is achieved due to heterogeneous vasculature formation found in pathological processes such as tumors, resulting in enhanced permeation retention effects. The accumulation of nano-drug delivery systems at the targeted site results in enhanced drug payload. **C.** In contrast, active targeting is achieved using recognition molecules attached to nano-drug delivery system surfaces for specific ligand-receptor mediated interactions.
Reprinted from [11] with permission from AAAS.

of nano–bio interactions, clearance and metabolic pathways and potential acute and chronic toxic effects of these nano-DDSs need to be addressed before their use in human can be approved.

2.1 Lipid-based nano-drug delivery systems

Since their initial introduction in the 1970s, liposome-based DDSs have successfully demonstrated the delivery of anti-cancer drugs, enzymes and proteins with numerous injectable formulations that are now used in clinical medicine [24]. So far, the many achievements obtained using liposomes have far surpassed all other nano-DDSs under investigation. Comprised of naturally-occurring biocompatible phospholipids and cholesterols, their successful bio-integration includes their non-toxic nature and the body's innate ability to metabolize them [25]. Versatile structural and physical properties of liposomes allow the development of a tunable DDS that can be synthetically manipulated by altering lipid composition, size and surface chemistry. In general, liposomes are classified based on two physical characteristics: size and the level of lamellarity. The three classes of liposomes include small unilamellar vesicles of 25 – 50 nm in size, large unilamellar vesicles and multi-lamellar vesicles consisting of several lipid layers separated by aqueous fluid [26]. Various physical properties of liposomes, including their robust stability, are determined by the lipid component of the liposomal bilayer. For example, the addition of excess cholesterols or the formation of a phospholipid with a high gel-liquid-crystalline transition temperature results in liposomes with an enhanced retention rate, minimizing the drug loss during bloodstream circulation [27]. Surface modification can also reduce drug loss from non-specific uptake. PEG conjugation to liposomal surfaces leads to prolonged circulation time by evading the reticuloendothelial system through the inhibition of opsonin binding on liposomal capsules [28]. In addition, active targeting of liposomes conjugated with antibodies or other ligands allows combined localized drug and contrast agent delivery to specific tumor sites (**Figure 3A** and **B**) [29]. Compared with many other types of nano-DDS, the major advantages of liposomes include biocompatibility, structural versatility, size and the ease of surface chemistry modification while accommodating a variety of therapeutic cargos regardless of their polarity and solubility. Robust drug release by liposomal DDSs can be achieved within intracellular or extracellular space through bilayer destabilization with demonstrated specific and generic treatment of heterogeneous tumors *in vivo* [30]. The development of lipid-based nano-DDSs with higher drug loading efficiencies, enhanced specificities and controllable drug release will improve the therapeutic index as well as reveal potential novel actions of some

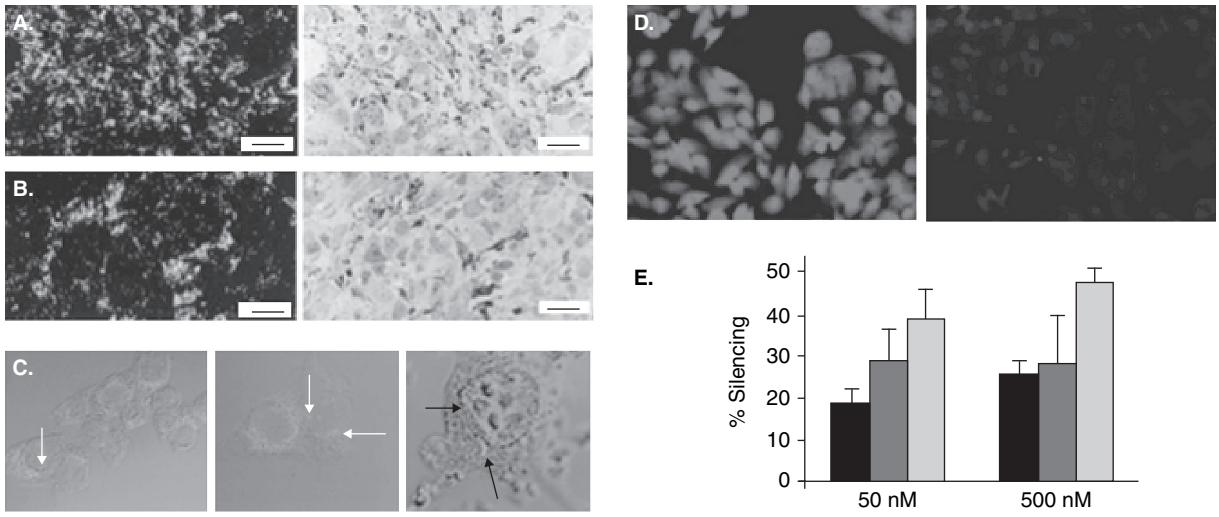

Figure 3. Soft matter and carbon-based nano-drug delivery systems. Anti-HER2 antibody conjugated liposomes showed extensive distribution within breast tumor BT-474 cells (**A**), whereas the unconjugated lipsosomes resulted in non-specific patchy accumulation around the tumor periphery (**B**). **C.** NIH 3T3 cells were transfected with fluorescent-labeled pDNA encapsulated biodegradable poly(lactic-co-glycolic acid) beads. Perinuclear fluorescence signals from the encapsulated membrane impermeable TOPO-1 probe demonstrate their uptake and localization inside cells. CNTs can be used as delivery vehicles for gene transfection. Gene silencing effects were observed upon treatment with CNT-siRNA, as demonstrated by the reduced expression level of lamin protein (**D**). **E.** CNT-siRNA (purple and grey) significantly improved gene silencing efficiency compared with the use of conventional transfection agent such as lipofectamine (blue).
A and **B** are adapted from [96], with permission from the American Association for Cancer Research.
C is adapted from [97], with permission from Nature Publishing Group.
D and **E** are from [98], with permission. Copyright (2005) American Chemical Society.
CNT: Carbon nanotube; siRNA: Short interfering RNA.

existing drugs, leading to a significant reduction in investment associated with new drug discovery research.

2.2 Polymeric nano-drug delivery systems

Polymeric nanoparticles for DDSs have garnered the interests of researchers for many decades. From the discovery of "Starburst" dendrimeric polymers in the 1980s to more recent advances in self-assembled polymeric micelles, various strategies have been developed to encapsulate drug molecules [31,32]. One of the most widely-studied polymeric nano-DDSs is using biodegradable systems for controlled and sustained drug release platforms [6]. A typical biodegradable nano-DDS consists of colloidal polymeric nanoparticles with drug molecules that are encapsulated, mixed, absorbed or attached onto the polymer matrix [25]. Various methods associated with drug loading, as well as polymer chemical properties directly regulate the mechanics of drug release. A main advantage of using these polymeric nano-DDSs is their biocompatibility and their sustained drug release capabilities at target-specific sites. Poly(D,L-lactide-co-glycolide) (PLGA), for example, has long been used as a starting material for bioabsorbable sutures. These polymeric structures breakdown in aqueous environments through hydrolysis, with resulting degradative byproducts metabolized through the Krebs cycle and subsequently eliminated by the renal system [33]. These PLGA carrier nanoparticles have been shown to intracellularly deliver therapeutic molecules for sustained drug release (**Figure 3C**). Labhasetwar and coworkers successfully demonstrated dexamethasone encapsulation within PLGA nanoparticles (using a double-emulsion technique) with subsequent cytoplasmic delivery [34]. Due to sustained drug level within the cytoplasm, an enhanced therapeutic effect was observed in DDS-treated smooth muscle cells, compared with those treated with free drugs, resulting from an efficient and prolonged interaction between the receptor for drug molecules [35].

In vivo demonstrations using these polymeric nano-DDSs also yielded promising results. Passive nano-DDS delivery to diseased tumor sites was achieved due to enhanced permeation and retention effects. Pathologic tumor blood vessels are dilated and porous, permitting both micro- and macromolecules to traverse through the endothelial lining of blood vessels, resulting in enhanced permeation. The retention of these diffused molecules is a consequence of absent functional lymphatic drainage within the malignant tumor bed, which further enhances nanoparticle accumulation. In contrast, active nano-DDS delivery relies on the attachment of cell- or tissue-specific targeting molecules to their surfaces [36]. Biomolecules such as peptides, oligonucleotides, oligosaccharides and antibodies are all potential candidates for active targeting. Using these mechanisms, biodegradable polymeric nano-DDSs enhance the localized and sustained delivery of drug molecules to diseased sites with minimal side effects (**Figure 2**).

Additional classes of polymeric nanostructures include self-assembled ampiphilic polymeric micelles, which have demonstrated high-stability in aqueous environments. Molecular self-assembled polymeric micelles composed of PEG–poly(α,β-aspartic acid) and PEG–poly(β-benzyl-L-aspartate) block co-polymer have exhibited high aqueous solubility and stability [5]. The core-shell structural characteristics allow for the encapsulation of hydrophobic drugs within their core, and targeting moieties can be attached around the outer shell. These block-copolymer micelles form spontaneously in aqueous solutions through the process of self-assembly when the polymer concentration is above the critical micellar concentration [37]. Drug molecules are loaded within the micellar core either through the physical encapsulation processes or by direct conjugation [32]. Typical therapeutic release mechanisms for drugs loaded within micellar structures include diffusion, micellar breakdown and covalent bond cleavage between drugs and micelles [38,39]. Structural design of micelles can dictate these kinetic processes, including the release rate of drug molecules. Intracellular delivery of encapsulated materials using self-assembled polymeric micelles has been well demonstrated. For example, Maysinger and coworkers showed that poly(caprolactone)-*b*-poly(ethylene oxide) block-copolymer micelles doped with fluorescent dyes readily undergo endocytosis in rat pheochromocytoma cells [40]. In addition, experimental studies using different polymeric precursors have been shown to deliver a variety of therapeutic agents. Poly(ethylene oxide)-*b*-poly(L-amino acid) micelles, for example, have been used to successfully encapsulate doxorubicin and cyclophosphamide with demonstrated enhanced anti-tumor effects *in vivo* [32]. However, more detailed characterization of immunocompatibility and adverse reactions are still needed to assess the usefulness of these polymeric DDSs.

2.3 Buckyballs and carbon nanotubes

The discovery of a new allotropic form of elemental carbon other than graphite and diamond in the 1980s resulted in the birth of fullerene research [41]. Spherical fullerenes, commonly referred to as buckyballs, not only won the 1996 Nobel prize in chemistry, but also played an instrumental role in the development of nanotechnology. Although the hydrophobic nature of fullerenes and their derivatives has been a major hurdle for their use in biomedical applications, more recent studies have shown that functionalized buckyball surfaces with hydrophilic groups such as carboxylic (-COOH) and primary amines (NH_3) have rendered them water miscible [42,43]. These water-soluble fullerene derivatives effectively penetrate through cell membranes and various biological barriers such as the blood-brain-barrier (BBB), which provide effective delivery of drugs to disease sites that are not accessible by conventional free drug [44]. In addition, several unique properties of buckyballs have demonstrated major potential for use in DDS platforms. For example, following an intravenous injection, buckyballs rapidly redistribute within various tissues and organs, which illustrates the highly efficient and robust nature of fullerene for alternative systemic drug delivery strategies [44]. Due to the presence of functional groups, chemotherapeutic drugs such as paclitaxel can be conjugated to buckyballs to increase the half-life of this drug by fourfold in bovine plasma [45]. Wilson and coworkers successfully linked buckyballs to moieties such as ZME-018 antibodies to target cell surface antigens (gp240) that are commonly over-expressed in human melanoma cells [46]. The successful demonstration of delivering chemotherapeutic and molecular drugs using functionalized buckyballs provides the fundamental principles for exploiting C-60 fullerenes for targeted drug delivery applications.

Cylindrical fullerenes, or carbon nanotubes (CNTs) have also shown great potential for use in biomedical applications [20]. Similar to buckyballs, CNTs are hydrophobic in nature. Therefore, the ability to use CNTs in biological research is highly dependent on the functionalization of their surfaces with biocompatible groups. Several strategies have emerged of late to render CNT more water-miscible. Strong acid-based oxidation of CNTs through reductive chemistry generates carboxylic groups on their surfaces [47]. Similar results have been achieved using diazonium chemistry to attach addends to the CNT side walls through covalent linkage [48]. These functionalized CNTs can be linked with active molecules including peptide, nucleic acid, proteins and therapeutic agents for targeted delivery and therapeutic purposes [20].

One of the main attributes of using CNTs as DDSs is their ability to efficiently enter cells. Several groups studied the transmembrane trafficking of functionalized CNTs in cells with various molecules attached to their surface [49]. Presently, the general consensus for CNT internalization is through passive uptake that is endocytosis independent. Results obtained using both theoretical calculations and experimental observations suggest that the rearrangement in the geometrical orientation of CNTs allows them to position perpendicularly to the plasma membrane and act as nanosized needles. CNTs enter cells through initially spearing the plasma membrane, followed by passively diffusing across the lipid bilayer [50,51]. Using this process, it is possible to design CNT-based DDSs that allow for an efficient uptake of drugs and therapeutic agents. For example, peptide-conjugated CNTs have been shown to stimulate an immune response in mice through complement system activation [52]. The delivery of plasmid DNA using functionalized CNTs has been shown to significantly improve the effectiveness of gene expression modulation, demonstrating the usefulness of CNTs as delivery vehicles for gene therapy applications (**Figure 3D** and **E**) [53]. Finally, although quite preliminary, functionalized CNTs have demonstrated their use as nano-DDSs: drug molecules such as amphotericin B were linked to CNT surfaces, and easily gained entry into

mammalian cells, with preserved antifungal activity against a variety of pathogens [54]. Although functionalized CNTs have been shown to efficiently deliver a wide range of therapeutic compounds into cells, delivery using *in vivo* systems remains elusive. Recently, Tagakaki and coworkers showed that carborane attached to CNTs resulted in higher targeted accumulation of boron within tumors, compared with the bloodstream or other organs [55]. The enhanced accumulation of boron atoms using functionalized CNTs provides evidence for using this system for boron neutron capture therapy in cancer management. Although a more complete biodistribution map and a better understanding of the cytotoxic effects are needed, these results show that CNT-based DDSs possess significant implications for cancer therapy.

2.4 Metallic nanostructures

Metallic nanostructures have long been used in biomedical applications such as biosensors for the detection of various antigens and DNA, as well as contrast probes for imaging, diagnostics and therapeutic platforms [56-59]. Of particular interest is in using colloidal gold nanoparticles as optical probes for biosensing and imaging applications. The confined collective electron oscillation gives rise to quantum mechanical effects such as surface plasmon resonance and Surface Enhanced Raman Scattering, which can be manipulated by changing nanoparticle size [57,60]. In addition, the relative ease in the ability to attach biomolecules onto their surfaces through various affinity interactions has gained considerable attention for their use in DDSs. Mirkin and coworkers functionalized gold nanoparticles with thiolated oligonucleotide sequences as antisense agents for gene therapy. The intracellular delivery of these antisense nanoparticle bioconjugates resulted in highly effective gene knock-down of the targeted mRNA sequence encoding for enhanced green fluorescence protein (EGFP) [61]. They reported much higher delivery efficiency compared with common gene transfection agents, including lipofectamine 2000 [61]. Similar gene delivery platforms using gold–nickel nanorod composites have been studied [62]. In addition, various groups have now shown the ability to conjugate antibody-based drugs for enhanced drug delivery. TNF, for example, was conjugated with PEGylated gold nanoparticles, resulting in enhanced anti-tumor activity [63].

Aside from using gold nanoparticles as direct carriers for drug delivery, their physical properties have also been exploited to control the amplitude and rate of drug release. West and coworkers incorporated gold nanoshells within hydrogel networks to selectively control the rate of drug release. By using gold nanoshells of different thickness, the dielectric properties of these materials were altered. These different nanoshells can generate heat upon optical excitation of various energies. Temperature elevation above the lower critical solution temperature resulted in the collapse of hydrogel networks with subsequent drug release into the surrounding diseased tissue [64]. Using gold nanorods, similar photothermically-induced volume transition in hydrogel networks was investigated as a possible strategy for controlled drug delivery platforms [65].

Other metallic nanostructures, such as magnetic iron oxide nanoparticles, have received significant recent interest. For example, magnetic nanoparticles incorporated within polymeric spheres have been encapsulated with therapeutic compounds [66]. In addition to conventional targeting using bio-recognition molecules (**Figure 4A**), magnetic nanoparticles can be used as a homing device to drugs and contrast agents to the targeted site by controlling the external magnetic field [67]. Once the desired destination is reached, polymer components degrade slowly to release the embedded drug molecules. Similar to gold nanoparticles, magnetic nanoparticles have also been used for the transfection of DNA sequences [68]. For example, intravenously injected magnetic nanoparticle-DNA complexes have been guided to a diseased site using a strong, high-gradient external magnet. Once the target site was reached, nanoparticle–DNA complexes diffused through tissues and enzymatic cleavage released the genomic sequence from the magnetic nanoparticles. The entire process is termed 'magnetofection' [69]. In addition, novel therapeutic agents such as small interfering RNA (siRNA) and antisense oligodeoxynucleotides have been successfully conjugated to magnetic nanoparticles, with a high level of success during 'magnetofection' procedures [69,70]. Despite the many developments using *in vitro* and small animal models, fundamental challenges using larger animals still remain. For example, to capture nanoparticle–DNA complexes that are further distances away from the magnet, a significantly higher magnetic field gradient is required. As high gradients lead to rapid decay of the field strength from the magnetic center, the use of presently-available rare earth magnets, such as NdFeB, cannot capture nanoparticle targets beyond a few centimeters away from the magnet [71].

Other inorganic nanoparticle systems have also attracted significant interest in using them as potential DDSs. Silica nanoparticles, for example, have demonstrated superior biocompatibility, stability and simple synthetic procedures. Various groups have demonstrated the successful loading of different therapeutic and contrast agents with silica nanoparticles for intracellular delivery applications. Roy *et al.* used surface functionalized silica nanoparticles to deliver DNA sequences for gene therapy [72]. Cells treated with silica nanoparticles conjugated to plasmid DNA encoding EGFP resulted in the expression of the *EGFP* gene, as observed from their green fluorescence emission. Using mesoporous silica nanoparticles, Slowing *et al.* showed that membrane-impermeable proteins such as the enzyme cytochrome c can be efficiently delivered within cells and maintain its enzymatic functionality [73]. Furthermore, Lu *et al.* have showed that by incorporating the chemotherapeutic drug camptothecin within mesoporous silica nanoparticles, they were able to induce cell apoptosis in a

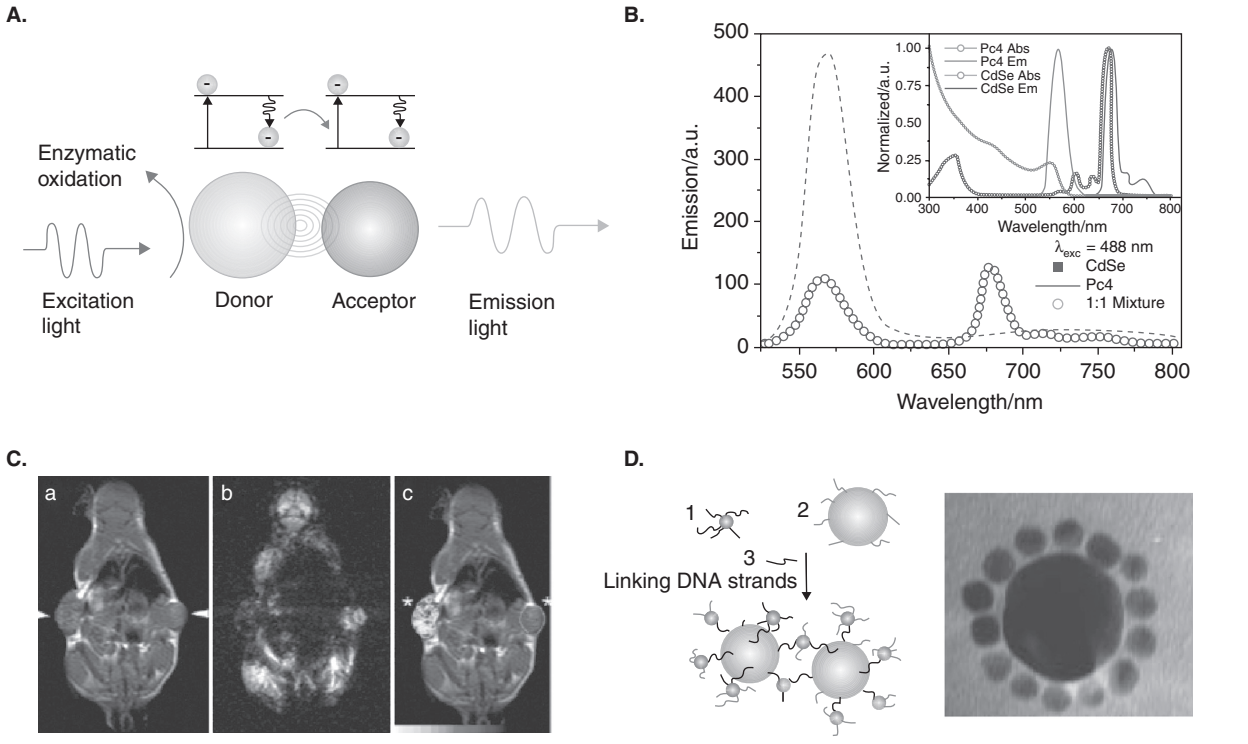

Figure 4. Semiconductor, metallic and hybrid nano-drug delivery systems. A. Qdots can function as electron donors to stimulate an electron acceptor in FRET. **B.** Exploiting this mechanism, Qdots were used as energy donors to generate singlet oxygen from classical photosensitizers such phthalocyanine. **C.** Magnetic nanoparticles conjugated to recognition molecules such as antibodies allow targeted delivery to diseased sites. *In vivo* MRI with transferrin-conjugated magnetic nanoparticles was used to analyze transferrin receptor expression level. **D.** Heterogeneous multifunctional nanostructures assembled using oligonucleotides and proteins to link semiconductors nanocrystals with metallic nanoparticles.

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FRET: Forster Resonance Energy Transfer; Pc4: Phthalocyanine; Qdots: Quantum dots.

variety of cancer cell lines [74]. In addition to delivery, the biocompatible nature of silica has also allowed researchers to explore possible means to modify other metallic and semiconductor nanomaterials for biomedical applications. Silica nanoparticle-modified metallic colloids, organic molecules and semiconductor nanocrystals are all been investigated as potential hybrid multifunctional delivery systems [75,76]. These promising results have paved the way for the eventual clinical adaptation of silica nanoparticle-based DDSs.

2.5 Hybrid multifunctional nanostructures

Metals and semiconductor nanomaterials have physical properties that can be manipulated for biomedical applications. For example, exposing metallic nanoparticles to electromagnetic radiation results in an enhanced localised electric field, due to collective electron motion [77]. This unique size-dependent property has been exploited to design Surface Enhanced Raman Scattering probes for diagnostic applications [78]. Semiconductor Qdots, on the other hand, have size-tunable fluorescence emission that spans the entire visible and near-infrared wavelength range, which makes them ideal candidates as optical-based contrast agents. Recently, more research has focused on the development of hybrid systems that integrate several different classes of nanomaterials to create a heterogeneous multifunctional platform for diagnostics, imaging and therapeutic applications. Semiconductor-based nanostructures, such as Qdots and quantum rods have gained vast attention in recent years as contrast agents for ultrasensitive biological imaging applications [79,80]. Advances in the surface modification chemistry of Qdots have allowed the present ability to prepare multifunctional systems that combine targeting and imaging modalities. So far, Qdots have been conjugated to a variety of biomolecules, including oligonucleotides, peptide sequences, proteins and engineered antibodies for *in vitro* and *in vivo* targeted labeling of cells, tissue and organs [81]. Recently, several groups demonstrated Qdots'

ability to generate reactive oxygen species and free radicals following long-term optical irradiation [82]. This discovery offered a potential means to use Qdots as photosensitizers in photodynamic therapy for cancer. Photosensitizers or photosensitive drugs are photosensitive molecules that can transfer light energy to surrounding molecular oxygen to generate reactive oxygen species (ROS). Following the absorption of photons, the sensitizers are transformed from the ground state to the excited triplet state. Reactive oxygen species are created via interactions between the excited sensitizers with surrounding biological molecules or oxygen through the transfer of an electron to create singlet oxygen [83]. ROS have been reported to induce tumor cell death through direct photo damage [84]. In addition, microvascular collapse from severe tumor tissue hypoxia and anoxia, as well as the activation of immune response through IL-10 and IL-6 up-regulation following ROS exposure, has been shown to contribute to the tumor killing effect of photodynamic therapy [85,86]. However, the effectiveness of photodynamic therapy largely relies on the ability of photosensitizers to generate ROS. Although the generation of singlet oxygen using a typical CdSe Qdots is much lower in comparison with classical photosensitizers [87], Qdots are much more stable against photobleaching, allowing prolonged irradiation of UV/visible light to potentially maintain similar levels of steady-state singlet oxygen generation. The ability to modify Qdot surface chemistry can be used as alternative means to attach classical photosensitizers to already functionalized Qdots. At close proximity to the Qdots, efficient energy transfer processes (such as Forster Resonance Energy Transfer) can occur (**Figure 4B**) [88]. Coupled with the ability to specifically target disease sites, Qdots can be used as energy donors, and classical photosensitizers as energy acceptors, to significantly enhance the effectiveness of photodynamic therapy (**Figure 4C**). In this sense, not only do Qdots act as DDSs for phototherapeutic drugs, they can also directly enhance their therapeutic efficiency through energy transfer processes.

Hybrid systems using both semiconductor and metallic colloids have also shown major promise. Mirkin and coworkers have demonstrated using complementary DNA sequences to effectively link Qdots onto colloidal gold nanoparticles to create satellite structures (**Figure 4D**) [22]. Similarly, Wang *et al*. covalently attached satellite structures using Qdots and magnetic iron oxide nanoparticles. Their system can potentially be used for multi-modal imaging platforms using both fluorescence and MRI technology simultaneously, as well as cell sorting under magnetic guidance [89].

These multifunctional systems can be utilized for therapeutic applications. For example, drug molecules can be attached to the surface of nanoparticles through covalent linkage. In addition, the development of organic–inorganic hybrid structures allow for the simultaneous encapsulation of drug molecules with contrast agents. Recently, Parak and coworkers used ampiphilic polymers as a universal approach for bio-functionalization of various nanocrystals, including $CoPt_3$, Au, CdSe/ZnS and Fe_2O_3. Expansion of these systems within drug molecules in the hydrophobic core of the capsule can lead to highly sophisticated DDSs with multiple modalities [90].

3. Expert opinion

The many developments in bionanotechnology represent tremendous progress over the past decade. Although conventional nanosized biomaterials such as liposomes and polymers have been approved for clinical use, newer classes of nanomaterials such as fullerenes, Qdots and metallic nanoparticles are many years away from reaching clinical trials. One of the key concerns among researchers and the general public is that of nanomaterial biosafety. In particular, carbon-based nanomaterials (such as fullerenes) and inorganic nanomaterials (such as Qdots) are typically known to be less biocompatible. Fullerenes for example, in their native form, have been shown to induce cellular cytotoxicity *in vitro* [91]. Semiconductor Qdots on the other hand are mainly composed of heavy metal ions such as cadmium, selenium and tellurium. The breakdown of these nanocrystals, either through oxidation or enzymatic degradation could result in the release of potent heavy metal ions. Derfus *et al*. demonstrated that cadmium ions released from CdSe Qdot degradation can cause significant hepatotoxic effects in liver cells (hepatocytes) primarily responsible for the body's detoxification process [92].

Various reports in the recent literature have been published to modify the surface chemistry of these nanomaterials to render them more biocompatible. For example, studies with CdSe Qdots have found that the additional coating of Qdots with inorganic ZnS and an organic polymer layer can significantly enhance their stability against environment-related stress [91]. Polymer-coated CdSe/ZnS core/shell Qdots are much more resistant to degradative effects, with minimal adverse cytotoxic effects observed in cell and animal models after 6 months of incubation [93,94]. However, long-term chronic nanomaterial exposure and the cumulative effects on biological systems remain to be elucidated. Fischer *et al*. showed, using CdSe/ZnS Qdots in rat models, that regardless of their surface coating Qdots are not eliminated from the body through fecal or urine excretions [95]. If the body lacks the ability to clear and eliminate these nanocrystals, where do they accumulate? Although the liver is the obvious choice for short-term sequestration, more studies have emerged demonstrating that alternative sites, including the bone marrow and lymph nodes could potentially act as reservoirs for these nanomaterials, whether in its crystalline or ionic forms [96]. Although far from complete, the studies involving nanocrystal pharmacokinetics can be used as potential model systems to characterize the mechanisms of biodistribution, sequestration

and clearance of other semiconductor, metallic and hybrid nanoparticles *in vivo*.

In addition to surface chemistry, the physical dimensions of nanostructures can also influence their biodistribution and clearance *in vivo*. Studies by Chithrani *et al*. showed that colloidal gold nanoparticles enter mammalian cells in a strict size and shape selective manner. For nanoparticles below or above a certain critical size of approximately 50 nm, the internalization process is highly inefficient [15]. In addition, Geng *et al*. have shown that polymeric systems of a certain shape have longer circulation time as compared to spherical nanostructures [16]. Although these findings are preliminary, they provide new insights into the possible connection between physical traits of nanomaterials and their *in vivo* distribution, sequestration and clearance of nanostructures.

The field of nanotechnology is a young and exciting discipline. The majority of work performed within the past decade has been based on proof-of-principle demonstrations of nanomaterials for biomedical applications. As this rapidly developing field begins to mature in the next decade, we anticipate more investigative commitments toward fundamental characterizations of nanomaterial–biological interactions. An understanding of how nanomaterials of various sizes, shapes, composition and altered surface chemistry interact with biological entities at the molecular, cellular, tissue and organ levels is crucial for their clinical implementation. Such tasks are not trivial, as it requires the collaboration and coordination of diverse disciplines involving material science, chemistry, molecular biology, immunology and pharmacy. Concurrently, government and funding agencies also need to take initiatives in this effort. According to the National Nanotechnology Institute, despite the US$1.3 billion US government funding for nanotechnology-related research in 2006, only a small fraction of the funding was allocated to fundamental studies on topics such as the biological and environmental impacts of nanomaterials [101]. Nanotechnology holds many promises, however, it is only through the fundamental understanding of the interface between nano-biological interactions that the true potential of nanotechnologies can be realized.

4. Concluding remarks

The advances in synthetic chemistry, molecular biology and material engineering have provided us with new sets of tools for biomedical applications. Of particular interest is the utilization of nanoscaled materials for DDSs. Nano-DDSs offer unprecedented advantages over conventional DDSs or free drug strategies due to their size, versatile surface chemistry and the ability to construct complex systems with multi-modal functionalities. These unique properties allow researchers to manipulate pharmacological and therapeutic properties to achieve higher drug efficacy while minimizing systemic side effects. In addition, implementing presently-available conventional drug formulations into these novel nano-DDSs can significantly reduce the associated expenditures in research and development related to drug discovery, which, at the present time, exceeds US$150 million for each new pharmaceutical formulation [3]. Finally, the integration of nanomaterials and therapeutic compounds allows for the creation of hybrid multifunctional systems that can simultaneously detect, image, diagnose and provide therapeutic interventions. Such complex system developments can only be achieved through the use of nanomaterials due to their unique optical, electronic and magnetic properties stemming from the effects of quantum confinement. Despite these promises, nano-DDSs also face many challenges and limitations. Toxicity is a major safety concern for using nano-DDSs in the clinical setting. However, with a better understanding of the biological interactions associated with nanomaterials and the improvements in surface chemistry modifications, it is not a matter of if, but when, such hurdles will be cleared. We can envision a future where tiny nano-DDSs travel through our bloodstream searching for sites of infection or disease processes, and once any irregular cellular activities are found, the triggered release of the nano-DDS' cargo repairs damaged tissues or kills foreign invaders. Every step along the way is meticulously executed in an intelligent automatic manner. This is the future of nanotechnology.

Declaration of interest

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