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Theranostic Nanoparticles Engineered for Clinic and Pharmaceutics

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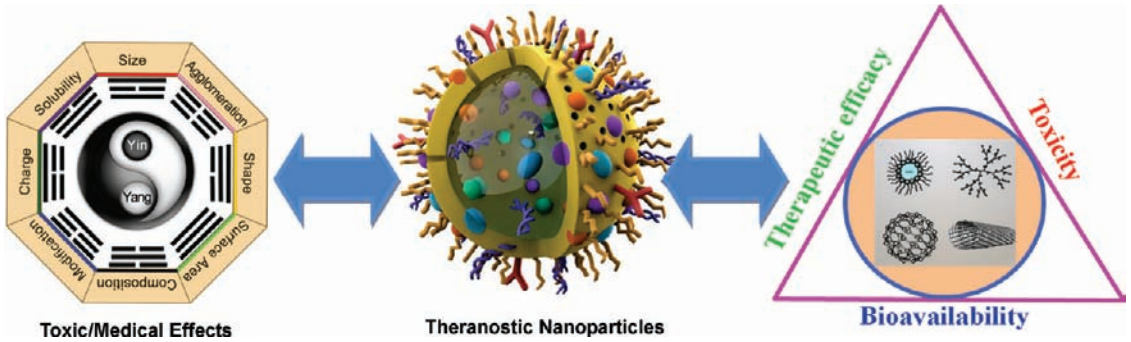

Nanomedicine is the manipulation of human biological systems at the molecular level using nanoscale or nanostructured materials. Because nanoscale materials interact effectively with biological systems, the use of nanodiagnostics and nanotherapeutics may overcome many intractable health challenges. A variety of nanoparticles have been designed with modifiable functional surfaces and bioactive cores. The engineering of nanoparticles can result in several advantageous therapeutic and diagnostic properties including enhanced permeation and retention in the circulatory system, specific delivery of drugs to target sites, highly-efficient gene transfection, and enhanced medical imaging.

These nanoscale materials offer the opportunity to detect chronic diseases early and to monitor the therapeutic effects of nanoformulated drugs used in the clinic. Many of these novel nanoparticles contain both drug(s) and imaging agent(s) within an individual nanoparticle for simultaneous disease diagnosis and therapy. Further integration of therapeutic compounds with diagnostic agents into theranostic nanoparticles would be highly beneficial.

However, the unique physiochemical properties that make nanomaterials attractive for therapy and diagnosis may be also associated with potential health hazards. Our research has demonstrated that the biological response to nanomaterials is related to many factors including exposure levels, systemic accumulation and excretion profiles, tissue and organ distribution, and the age of the test subject. Therefore, when engineering new nanomaterials for clinical use, researchers need to consider these factors to minimize toxicity of nanoparticles in these applications. We have fabricated and evaluated nanomaterials such as cationic amphiphilic polymers and metallofullerenes that demonstrate both high efficiency and low toxicity in gene therapy and/or chemotherapy. In this Account, we describe the development of theranostic nanomaterials with low toxicity and illustrate their potential use as novel nanomedicines in translational research.

1. Introduction

With the emergence of nanotechnology, we have acquired new methods for manipulating materials on the atomic or molecular scale for a variety of applications. The unique properties of engineered nanomaterials endow them with dynamic physiochemical features different from those of bulk materials with the same compositions. These unique properties are very attractive for pharmaceutical and clinical applications. Engineering of nanoparticles for combined therapeutic and diagnostic applications (theranostic nanoparticles) requires knowledge of their material, chemical, physical, biochemical, and toxicological properties and is under intense investigation.

Various new tools for biomedical research and clinical applications have emerged with recent advances

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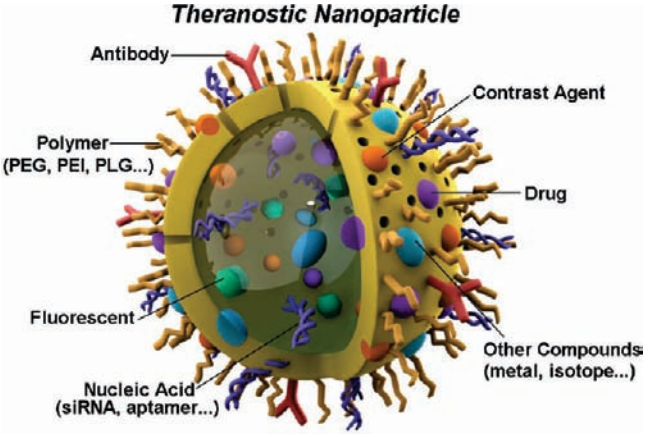

FIGURE 1. Theranostic nanoparticles can be surface functionalized with antibody and polymers to achieve targeted delivery and improved biocompatibility. The interior core of nanoparticles can be encapsulated with various bioactive compounds, such as nucleic acids, imaging contrast agents, drugs, and fluorescent materials to fulfill different theranostic purposes. Molecules are not shown to scale.

in nanotechnology. The surface of nanoparticles can be modified to achieve targeted delivery and improved biocompatibility. Compounds may also be encapsulated within the interior core of nanoparticles for multiple functions (Figure 1). A number of nanomaterials have shown promise as drug and gene delivery vehicles as well as diagnostic agents. For example, iron oxide nanoparticles are known superparamagnetic materials which serve not only as magnetic resonance imaging (MRI) contrast agents for *in vivo* imaging but also as vehicles for manipulation by magnetic fields to improve drug delivery or to allow for localized heat therapy.[1] Along with this significant progress in the medical uses of nanoparticles, concerns about their potential toxicities have been raised and must be addressed in designing nanomedication systems.

The unique material characteristics of nanoparticles, including high surface area, facile surface modification, small size, and novel magnetic or optical properties, make nanoparticles promising candidates for biomedical applications. This Account gives an overview of selected recent developments and medical applications of potential theranostic nanoparticles.

2. Low Toxic Nanoparticles Designed as Drug and Gene Delivery Vehicles

Cellular delivery involves the transport of various drugs and biomolecules. Cargos carried by nanoparticles can be protected from enzymatic degradation which often accompanies absorption and distribution. Also, drug solubility and intestinal permeability often limit the oral bioavailability of potential drugs. As a solution to this problem, hydrophobic drugs can be incorporated into nanoscale drug delivery vehicles and transported into cells. This ability allows us to reexamine promising drugs previously dropped from development due to poor solubility.

During the past decade, liposomes, micelles, and nanoemulsions have been developed as drug and gene delivery systems. Abraxane, a nanosized albumin-bound paclitaxel emulsion, was approved by the U.S. Food and Drug Administration for the treatment of metastatic breast cancer in 2005. Fewer allergic reactions were observed in patients using this nanoformulation compared to free paclitaxel. A nanoemulsion of docetaxel (ANX-514) showed bioequivalence and overall safety comparable to the Taxotere formulation of docetaxel. In addition, other drug and gene delivery systems based on polymers, dendrimers, as well as biomolecules are also at different stages of preclinical and clinical development. For example, compared with a standard paclitaxel formulation, ABI-007, a nanoparticle formulation of paclitaxel which is cremophor-free, can be more efficiently and safely administered at high doses with superior response.[2] In clinical trials of this nanoparticle formulation, prolonged survival time with no severe hypersensitivity reactions was observed when treating metastatic breast cancer.[2] Development of nanodrug and gene delivery systems requires an understanding of the absorption, distribution, metabolism, and excretion profiles of the nanomaterials. This clinical profile is required not only to optimize the clinical effects of nanomaterials but also to provide guidance for their safe use.

2.1. Nanoparticles Constructed as Drug Carriers for Efficient Delivery. Nanosized carrier systems have the potential to prolong the half-life of the encapsulated drug in the body through enhanced permeation and retention (EPR) effects. A number of nanoparticle-mediated effects, such as improved chemical stability, controlled release from the nanoparticle, and protection of the drug from the immune system, may result in prolongation of a drug's half-life and increased therapeutic window.

Incorporation of drugs into nanodelivery vehicles has resulted in a new paradigm for lowering the adverse effects of chemotherapeutic drugs. Broad application of paclitaxel and doxorubicin is limited by their physiochemical properties that result in intolerable side effects. The replacement of paclitaxel's solubilizer, Cremophor, with amphiphilic cyclodextrin nanoparticles prevents paclitaxel from undesirable recrystallization in aqueous solutions and significantly reduces the drug's side effects such as hemolysis and cytotoxicity.[3] When doxorubicin is loaded into biodegradable

poly(D,L-lactide-*co*-glycolide) nanoparticles for oral chemotherapy, it exhibits not only reduced cardiotoxicity compared to free doxorubicin but also improved oral bioavailability.[4]

One of the major challenges in drug delivery is selectively targeting diseased tissues. The efficacy of cancer chemotherapy is greatly limited by the incidence of toxicity to healthy tissues, attributed to the lack of specificity exhibited by anticancer agents for cancerous cells. Recent research has led to development of nanocarrier systems for delivery of anticancer drugs with improved therapeutic efficacy and reduced side effects. A ferrocenyl diphenol tamoxifen derivative, incorporated into lipid nanocapsules, shows antiproliferative activity specific for malignant glioma cells, but it demonstrates low toxicity levels in normal brain cells.[5] Xu et al. developed biocompatible solid lipid nanoparticles for the specific delivery of docetaxel to hepatoma cells. Targeted delivery was achieved in this case by using a galactose moiety that recognized an asialoglycoprotein receptor upregulated on the hepatoma cells' surface. While increasing cellular uptake by hepatoma cells and drug accumulation in tumor, this targeted nanocarrier of docetaxel is well-tolerated *in vivo*, without impairing liver function, as observed histologically.[6]

For anticancer drugs to be effective, it is just as important to appropriately release the drug from the nanocarriers as it is to deliver the drug to the malignant cells. Controlled release of drugs from nanoscale formulations has been demonstrated. For example, encapsulation of Temozolomide, a drug used to treat brain tumors, in solid lipid nanoparticles resulted in sustained-release of Temozolomide, while avoiding the adverse side effects such as cardiac and nephric toxicity caused by the conventional formulation.[7] Our group has been successfully engineered nanomaterials to control drug release. By using a dually pH- and temperature-responsive poly(*N*-isopropylacrylamide)-*co*-acrylic acid (PNiPAM/AA) hydrogel cage to cover the mesopores of silica nanospheres, it was possible to avoid the leakage of toxic isoniazid, an antitubercular drug, from the nanosphere. The bioresponsive properties of this nanosphere enhanced control of drug release.[8] Nanoparticle-mediated drug delivery systems such as ammonium-glycyrrhizinate-loaded chitosan−poly(acrylic acid) (CS−PAA) polymer magnetic nanoparticles were also used to treat infectious diseases by our group. These polymer magnetic nanoparticles showed unique pH-dependent behaviors on the size and zeta potential. CS−PAA polymer nanoparticles are amphiphilic and biodegradable and act as drug carriers with high loading efficiency and continuous release of the entrapped ammonium glycyrrhizinate.[9]

Anatomical and physicochemical barriers in the lung make delivery of drugs a challenge in pulmonary medicine. Nanotechnology has provided novel approaches for pulmonary drug delivery resulting in improved local and systemic bioavailability of drugs. In lung transplantation recipients, liposomal delivery of lipophilic immunosuppressants such as tacrolimus has provided sustained release and less frequent administration of the drug, and resulted in reduced dose-related toxicity.[10] Tam et al. demonstrated that an aerosolized nanoparticle formulation of the hydrophobic immunosuppressant, amorphous cyclosporine A, has an enhanced dissolution rate and, therefore, increased drug penetration and diffusion into lung tissue and the bloodstream. In addition, this nanoparticle formulation does not cause lung tissue irritation which is a frequent problem when using a solution-based pulmonary formulation.[11]

In general, the two major challenges hampering ophthalmic drug delivery are the unique anatomical and physiochemical barriers of the eye and rapid precorneal drug loss. Liposomes and nanoparticles have been used to improve corneal penetration and achieve controlled delivery and sustained drug release. A biocompatible polymeric nanoparticle suspension loaded with sodium diclofenac has been developed. This suspension does not irritate ocular tissues *in vivo* and has a favorable mean size for ophthalmic applications.[12] Kao et al. successfully incorporated pilocarpine into chitosan/carbopol nanoparticles for ocular applications. These nanoparticles showed little toxicity and a better prolonged release profile compared with pilocarpine in solution, gel, or liposomes.[13] The easily modified characteristics of chitosan-based nanosystems make them suitable candidates for ocular nanomedicines.

Nanoparticles can cross biological membranes in a nondisruptive way without apparent toxicity. This property makes nanoparticles particularly useful for increasing drug bioavailability in brain. Superoxide dismutase, a scavenger of reactive oxygen species, poorly penetrates the blood brain barrier (BBB). However, when encapsulated in biodegradable poly(D,L-lactide-*co*-glycolide) nanoparticles, superoxide dismutase was observed to cross the intact BBB and efficiently reduce damage caused by cerebral inchemia−reperfusion.[14] In combination with focused ultrasound, which can locally and transiently disrupt the BBB, magnetic nanoparticles carrying chemotherapeutic agents can sufficiently penetrate the BBB and accumulate in brain both passively and actively, as monitored by MRI.[15]

2.2. Nanomaterials Employed as Nucleic Acid Vehicles for Effective Gene Therapy. Gene therapy is a promising new approach for treating a variety of genetic and acquired diseases. However, these unstable macromolecules have poor cellular uptake and can be rapidly degraded by nucleases. To overcome these limitations, various chemical modifications of oligonucleotides have been tried. These modifications, however, have disadvantages such as decreased mRNA hybridization, elevated cytotoxicity, and increased nonspecific targeting. Therefore, there is a critical need to develop oligonucleotide delivery systems which protect these oligonucleotides from enzyme degradation and provide enhanced intracellular transfection efficiency in targeted cells. Viral vector systems have been generally used, owing to their superior ability to deliver and express genes to the targeted cells. However, their shortcomings, such as immunostimulation, small transgene size, high cytotoxicity and cost, limit the application of viruses in gene delivery.

In order to overcome the disadvantages of viral carriers, nonviral carriers have been designed as alternative systems. The advantages associated with nonviral carriers include facile large scale manufacture, low immunogenic response, versatile modifications, and the capacity to carry large inserts.[16] Amphiphilic and biodegradable cationic copolymers are efficient gene delivery systems which can condense nucleic acid and form controlled nanosized complexes.[17,18] Polyamidoamine (PAMAM) and poly[2-(dimethylamino)ethyl methacrylate] (PDMAEMA) are low toxic polymers which have shown great potential as ideal carriers because of versatile strategies for their synthesis using atom transfer radical polymerization (ATRP) or reversible addition−fragmentation chain transfer (RAFT) polymerization methods. Polycaprolactone (PCL), a hydrophobic biodegradable polyester which can be synthesized by ring-opening polymerization (ROP) of ε-caprolactone, is another promising delivery system. PCL and PDMAEMA have been combined to construct the amphiphilic carriers, such as PCL/PDMAEMA diblock or triblock copolymers,[19] PCL-*graft*-PDMAEMA copolymers, and its PEGylation derivations.[20]

Our group has synthesized amphiphilic copolymers by ROP and ATRP. PCL-*g*-PDMAEMA graft copolymers self-assembled into core−shell nanoparticles, composed of hydrophobic PCL and hydrophilic PDMAEMA with an ultralow critical association concentration of 8.1×10^4 g/L^{-1}. The resulting PCL-*g*-PDMAEMA nanoparticles have strong nucleic acid condensation ability. *In vitro* gene transfection studies indicated that PCL-*g*-PDMAEMA nanoparticles presented higher efficiency than Lipofectamine 2000 and the polymeric materials gold standard PEI.[21] It was determined that PCL-*g*-PDMAEMA nanoparticle/DNA complexes could escape from the endosome and release their payloads effectively in cytoplasm, which may be induced by the enhanced interaction between the complexes and cell membrane due to hydrophobic modification.[22,23] These cationic amphiphilic PDMAEMA nanoparticles have the ability to entrap hydrophobic drug and nucleic acid payloads simultaneously, which also exhibited excellent gene transfection efficiency and pH responsive sensitivity for releasing encapsulated drug payload.

Small interfering RNA (siRNA) has attracted much attention because it enables sequence-specific manipulation of expression for multiple endogenous genes. However, compared with DNA, RNA is more unstable due to structural differences and the ubiquitous presence of RNase. Factors limiting the gene silencing extent and duration include limited permeability of siRNA across cell membrane, low *in vivo* stability, stimulation of innate immune responses, and substantial liver and renal clearance. Using nanoparticles as siRNA carriers to transfer siRNA while retaining its bioavailability has been an active area in the nanodrug carrier research field.

Thermal, pH, and redox responsive polymer conjugates have been engineered to efficiently load and transfer siRNA into cells. The intracellular release of siRNA from pluronic/poly(ethylenimine) nanocapsules was achieved by changing the nanocapsules from a collapsed state to a swollen state using a brief cold shock treatment.[24] Meyer et al. synthesized a pH and redox sensitive endosomolytic poly(ethylene glycol)−polylysine−dimethylmaleic anhydride−melittin−siRNA polymer conjugate. Release of siRNA within the cell was triggered by changing the pH and adding reducing agents.[25] The first targeted delivery of siRNA was administrated in humans for solid tumor treatment.[26] This delivery system was a self-assembling cyclodextrin polymer-based nanoparticle denoted as CALAA-01. In this system, nonchemically modified siRNA was well-protected in the cyclodextrin polymer and intravenously administered to patients.

Dendrimers are nanostructures increasingly employed as nucleotide and drug delivery vehicles due to their exceptional intrinsic properties such as highly branched and globular shape, ease of synthesis, monodispersity, well-defined molecular weight, and precise number of peripheral groups that can be functionalized with bioactive molecules.

The low transfection efficacy of siRNA into HIV-susceptible cells limits the success of RNAi mediated therapy for HIV infection. Weber et al. reported an amino-terminated carbosilane dendrimer-bound siRNA delivery system. These RNase-resistant carbosilane/siRNA dendriplexes have a high and prolonged gene-silencing effect, and can be safely used in serum and antibiotics containing medium, without affecting cell viability and metabolic activity at relatively high dendrimer concentrations.[27] Poly(amidoamine) dendrimers have been extensively explored as genetic transfection agents. However, their relatively high cytotoxicity is related to the nonspecific interaction between the dendrimer's positively charged surface amine groups and the cell membrane. Internally quaternized and surface-acetylated poly(amidoamine) dendrimers exhibit reduced cytotoxicity due to their modified neutral surface and internal cationic charges which protect siRNA from enzymatic degradation. This nanocarrier can facilitate siRNA's penetration into the targeted cells, resulting in a homogeneous distribution of siRNA inside the cytoplasm.[28]

One of the most common methods used for the systemic delivery of siRNA involves their electrostatic interaction with cationic liposomes. Self-assembled liposome–protamine–hyaluronic acid nanoparticles modified by DSPE-PEG with conjugated ligand have been used to overcome innate immune responses of siRNA-based therapy. The developed nanoparticle formulation has a siRNA encapsulation efficiency of 90% and showed very little systemic immunotoxicity or organ damage over a wide therapeutic window.[29] Combined with low-frequency ultrasound treatment, a novel cationic nanoliposome can stably encapsulate, protect, and deliver therapeutic siRNA B-Raf and Akt3 to melanoma cells. As a result, cooperative inhibition of protein expression and consequent retardation of early or invasive cutaneous melanoma was observed with negligible associated systemic toxicity.[30]

3. Low Toxic Nanoparticles Developed for Diagnosis and Therapeutics

Many newly fabricated nanomaterials have displayed favorable therapeutic and diagnostic properties in research, implying their enormous potential as medicinal candidates. For example, iron oxide nanoparticles, due to their well established magnetic properties, are broadly used as contrast agents and can generate much heat in an alternating magnetic field. When iron oxide nanoparticles are surface-modified with bovine serum albumin, they have been found to selectively target the cancer cells and kill them thermally. This provides a minimally invasive way to treat tumors not eligible for surgical removal.[31] Gold nanoparticles are especially attractive for diagnosis and therapy due to their unique surface plasmon resonance effects, enhanced light scattering and absorption ability. It has been demonstrated that Au-based nanoparticles as photothermal agents can selectively destroy SK-BR-3 breast cancer cells in photothermal ablation therapy.[32] The use of molecular imaging techniques permits quantifying molecular changes associated with the onset and development of pathologic states. This approach can provide early diagnosis and prognosis of diseases like cancer. The imaging of cellular and subcellular structures requires imaging agents having high relaxivity density and specificity for targeting specific cells and tissues. Currently, MRI is still one of the most important diagnostic applications of magnetic nanoparticles as contrast agents. PEG-coated iron-oxide–gold core–shell nanoparticles (PEG-AuIONs) are promising MRI contrast agents for diagnosis of malignant tumors,[33] and core/shell structured iron (Fe)/gold (Au) NPs, prepared by the reverse micelle method, exhibited a high relaxivity as MRI contrast agents.[34]

Since their discovery in 1985, fullerene and fullerene-based derivatives have received intense interest due to their unique physiochemical properties. Functionalization of the surface of fullerenes frequently results in dramatic changes in their biological properties, making these compounds extremely versatile. Nano-C60 is known to produce reactive oxygen species (ROS) with high efficiency following photoactivation and cause brain and neuron toxicity. However, many fullerene derivatives are reported to be cytoprotective agents through scavenging free radicals and thereby reducing mitochondrial damage.[35] Fullerene derivatives was also reported to efficiently inhibit the reverse transcriptase activity of human immunodeficiency virus, to inhibit tumor cell proliferation, and to be potentially used as novel therapeutic agents.[36] For example, pretreatment by fullerenol has been reported to reduce radiotherapy-induced oxidative stress *in vitro* and enhance mitochondrial superoxide dismutase and glutathione peroxidase activity.[37] Sameh et al. studied the physiological functions of a tris-malonic acid derivative of the fullerene C_{60} molecule (C_3). Their results show that it functions similar to mitochondrial manganese superoxide dismutase (MnSOD) and can remove superoxide radical, a tissue damaging byproduct of mitochondrial electron transport. Treatment with fullerene C_{60} increased the life-span of MnSOD deficient mice by 300%.[38]

Our group has synthesized gadolinium metallofullerenes $[Gd@C_{82}(OH)_{22}]n$ having the transition metal atom

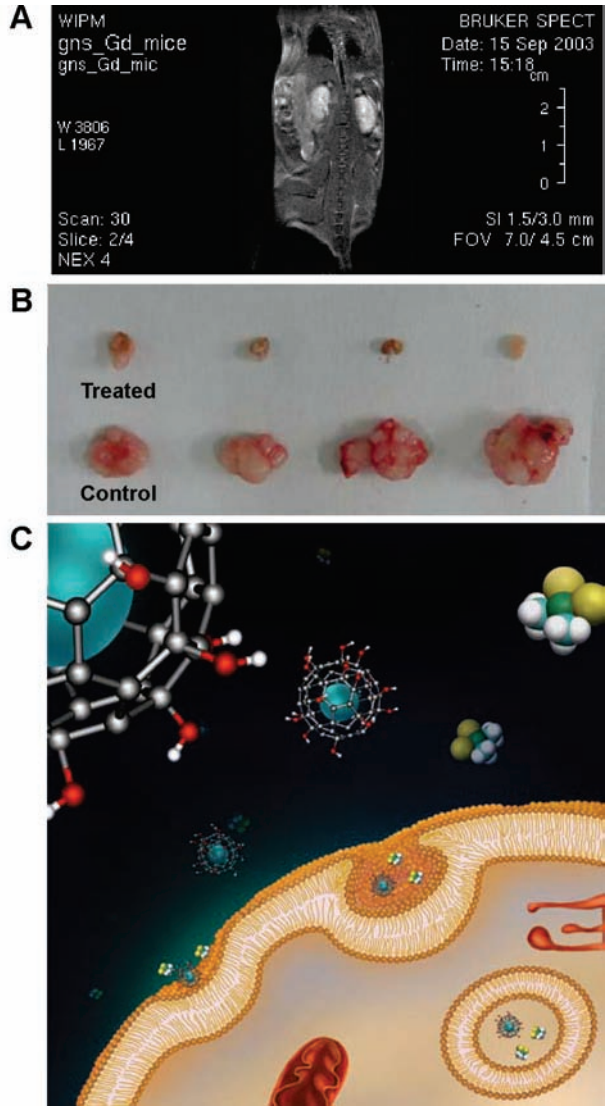

FIGURE 2. Nanoparticles are designed for diagnosis by molecular imaging and effective therapy due to penetration−retention in the tumor region. (A) 12× MRI relaxivity *in vivo* is achieved for administration of [Gd@C_{82}(OH)$_{22}$]n nanoparticles in doses of 6.5 μmol/kg, better than that of Gd-DTPA at ∼130 μmol/kg mice body weight. All MR imaging was carried out at 25 °C on a Bruker 4.7 T/30 cm Biospec magnetic resonance imaging scanner. (B) Treatment of [Gd@C_{82}(OH)$_{22}$]n nanoparticles inhibits tumor growth in mice. (C) Nanoparticles activate endocytosis in the human chemotherapy-resistant cancer cells. The metallofullerene nanoparticles [Gd@C_{82}(OH)$_{22}$]n may circumvent cancer cells' resistance to chemotherapeutical drugs by nanoparticle-enhanced endocytosis, thus increasing intracellular drug concentration.[44]

gadolinium (Gd) encapsulated in a C82 fullerene cage. It was originally designed as a desirable contrast agent for magnetic resonance imaging.[39] A 12× MRI relaxivity was observed *in vivo* for [Gd@C_{82}(OH)$_{22}$]n nanoparticles with ordered microstructures compared to commercial Gd-DTPA (Figure 2A). Since surrounding water molecules will not directly interact with the gadolium metal inside the fullerenol cage, the higher relaxation rate is ostensibly due to electronic interactions between the water molecules and paramagnetic cage of the metallofullerenol. The large surface area of the paramagnetic fullerenol cage, interacting with numerous water molecules simultaneously via hydrogen bonding, makes the spin−lattice relaxation process faster and the T1 relaxation time shorter. The size of [Gd@C_{82}(OH)$_{22}$]n nanoparticles is about 65 nm, which allows them to easily escape the reticuloendothelial system uptake *in vivo*. When these nanoparticles are administrated to tumor-bearing mice for molecular imaging, it was found that [Gd@C_{82}(OH)$_{22}$]n nanoparticles could penetrate through the vascular endothelial system and localize in tumors. Importantly, tumors were found to shrink due to the retention of [Gd@C_{82}(OH)$_{22}$]n nanoparticles (Figure 2B). Recent research has shown that this endohedral metallofullerene has additional promise as a chemotherapeutic agent. [Gd@C_{82}(OH)$_{22}$]n was found to strongly inhibit the growth of hepatoma cells (H_{22}) in mice and human breast cancer cells in nude mice. Much lower doses of [Gd@C_{82}(OH)$_{22}$]n were required compared to conventional antineoplastic drugs.[40] [Gd@C_{82}(OH)$_{22}$]n has a strong capacity to improve cellular immune processes by stimulating T cells and macrophages to release significantly greater quantities of Th1 cytokines and inducing the maturation of dendritic cells,[41] protect normal cells by scavenging ROS and inhibiting lipid peroxidation *in vivo*,[42] and specifically decrease the tumor microvessels density and lower the speed of blood supply to tumor stroma without effecting normal capillary vessels.[43] Development of drug-resistance to chemotherapeutic agents, such as cisplatin, is a major limitation on the success of chemotherapy. Our group has demonstrated that when this novel nanomaterial is coadministered with cisplatin, enhanced endocytosis of cisplatin is achieved. Thus, the presence of [Gd@C_{82}(OH)$_{22}$]n reactivated the impaired endocytosis of cisplatin-resistant cells. A resulting increase in intracellular drug concentration and formation of cisplatin−DNA adducts was observed (Figure 2C).[44] Toxicological diagnosis proved there are no clearly pathological changes between the nanoparticle treated group and control group at therapeutic doses (Figure 3).

4. Functionalization for Safe Theranostic Nanoparticles

Nanomaterials with different sizes, charges, and compositions have been broadly employed in drug and nucleic acid delivery as well as treatment of human diseases. However,

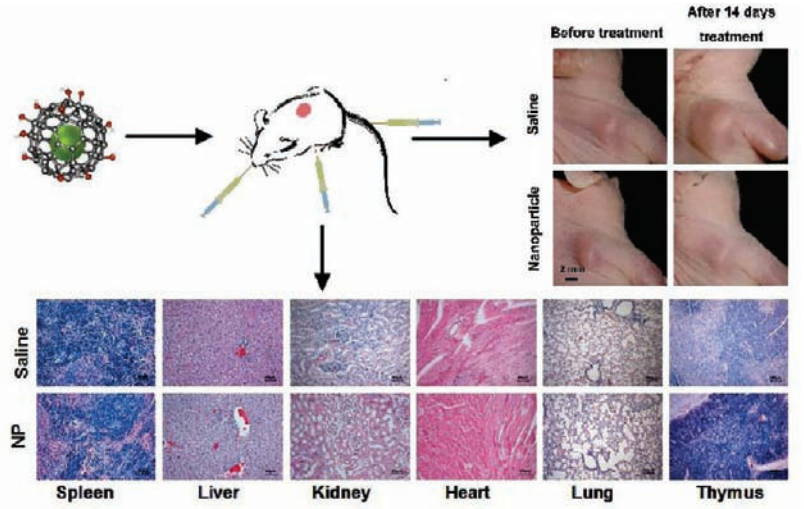

FIGURE 3. Tumor growth was inhibited in nude mice by [Gd@C_{82}(OH)$_{22}$]n nanoparticles. The tumor size of mice treated with nanoparticles was much smaller than that of control animals.[42] Hematoxylin and -eosin staining of the spleen, liver, kidney, heart, lung and thymus tissues from the nanoparticle-treated mice under therapeutic doses shows that the nanoparticles did not cause serious injury to these organs.[44]

the unique physiochemical properties that make nanomaterials so attractive may be potentially associated with hazardous health impacts. Nanoscale vehicles consisting of metal components may induce hepatocytic necrosis and renal proximal tubule necrosis *in vivo*. Acute toxic responses such as lesion of spleen, pulmonary vascular thrombosis, renal glomerulus swelling, and hepatic fibrosis have been associated exposure to metallic oxide nanoparticles. Our research demonstrates that the biological response to nanomaterials is related to many factors, including exposure concentration, systemic accumulation and excretion time, tissue and organ distribution, the age of the individual organisms, and so forth. Therefore, factors influencing toxicity must be carefully considered when engineering novel nanomaterials for use in diagnosis and therapy.

Concerns about unexpected toxicity must be addressed early in the engineering of nanomaterials for medical uses. We have described nanomaterials having low toxicity and great promise for use in chemotherapy, gene delivery, and siRNA delivery. Nanomaterials can be modified in a number of ways to reduce their toxicity. Properties of nanoparticles which can be modified to reduce toxicity are summarized in Figure 4. "Yin" (toxic effects) and "Yang" (medical functions) of a nanoparticle represented with a Chinese traditional "Taiji" ball are mutually modulated by the nanoparticle's significant physical and chemical properties including the particle size, aggregation state, shape, surface area, composition, surface chemistry, surface charge, and solubility. For example, toxic effects of nanoparticles can be moderated by changing the composition of the nanomaterials or

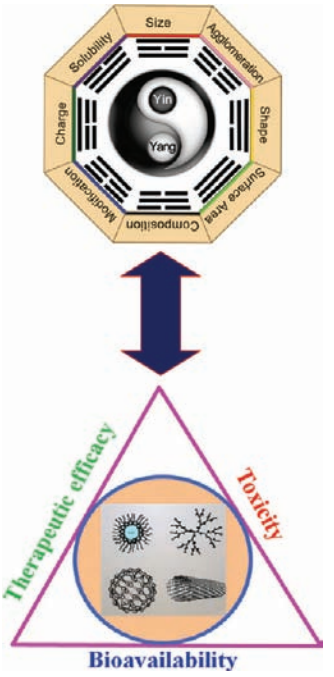

FIGURE 4. Schematic of components needed for integrated diagnostics and therapeutic (theranostic) nanoparticles. The nanoparticle is considered as being similar to the "Taiji" ball in Chinese philosophy. A nanoparticle's toxic or medical characteristics perfectly match the "Yin" and "Yang", two elements of the ontological necessity of "Taiji". While "Yin" (toxic effects) and "Yang" (medical functions) are two contrary aspects of nanoparticles, they can be mutually converted by modifying the nanoparticle's features, including the particle size, aggregation state, shape, surface area, composition, surface chemistry, surface charge, and solubility. Therefore, "Yin" and "Yang" can be reconciled and both belong to the same sphere of discourse. Integrated theranostic nanoparticles are designed to treat diseases while monitoring the therapeutic efficacy, toxicity, and bioavailability of the drug simultaneously.

modifying the nanomaterials' shape (e.g., rod, cube, ball, etc.). These modifications can affect the half-life of the nanomaterial and/or uptake by the targeted cells. It is important to utilize each property shown in Figure 4 when engineering nanoparticles. Modifying these features can result in reduction in toxicity and production of nanoparticles having expanded and multiple applications. The resulting theranostic nanoparticles enable us to simultaneously monitor the therapeutic efficacy, toxicity, and bioavailability of the drug with diagnostic imaging when treating diseases.

5. Future Perspective

Major challenges remain for developing nanomaterials for clinical diagnosis and therapy. These include (1) developing simple and controllable chemical methods for modifying the surfaces of nanoparticles so that toxicity is minimized; (2) understanding the biochemical mechanisms through which nanoparticles function *in vivo* (important mechanism details include determining how nanoparticles target certain tissues and factors that affect release of chemical payloads of nanoparticles *in vivo*); (3) utilizing the unique properties of nanostructures/nanomaterials to develop a new generation of medicine called "theranostic medicine" that possesses revolutionary advantages compared to the "traditional medicine"; (4) quantifying nanoparticles in a specific organ *in vivo* (this is of particular importance for understanding distribution of nanoparticles in the body); (5) identifying how the nanoparticles change in biological microenvironments, which directly relates to their transformation and metabolism behaviors; (6) understanding the toxic responses, particularly the long-term toxic effects of medical nanoparticles in the body. Finally, it is important to note that, in order to realize the intriguing dreams of nanomedicine for the most effective therapy and diagnosis of human diseases, we need multidisciplinary knowledge and techniques from nanochemists, nanophysicists, medical scientists, clinical doctors, nanotoxicologists, and so forth to construct safe and effective theranostic nanomaterials. Although more research is needed before theranostic nanomedicine can be routinely implemented in the clinic, current research indicates that theranostic nanomedicine may revolutionize the diagnosis and treatment of many illnesses.

BIOGRAPHICAL INFORMATION

Xiaowei Ma is a graduate student studying at the Division of Nanomedicine and Nanobiology, National Center for Nanoscience and Technology. She is now working on the biological effects of metallic nanoparticles.

Yuliang Zhao is Professor in Chemistry and Physics. He moved to Chinese Academy of Sciences from RIKEN (Japan) as a Hundred Elite Professor in 2001. He is mainly focused on the biomedical effects of nanostructure/nanoscale materials, including: (1) the biomedical functions of manufactured nanomaterials, (2) the toxicological effects of nanomaterials and establishing standard procedures for safety assessment of nanoproducts, (3) surface chemistry of nanoparticles and their novel properties, and (4) molecular dynamics using theoretical simulation and modeling the dynamic processes of the interplay between nanosystems and biosystems.

Xing-Jie Liang completed his postdoc at the Center for Cancer Research, NCI, NIH, and worked as a Research Fellow at Surgical Neurology Branch, NINDS. He focused on molecular imaging at the School of Medicine, Howard University before he worked as a Hundred Elite Professor at Chinese Academy of Sciences. He is a founding member of International Society of Nanomedicine and an editorial board member of *Acta Biophysica Sinica* and *Current Nanoscience*. Developing drug delivery strategies for prevention/treatment of AIDS and cancers, based on a fundamental understanding of the physiochemical and biological processes in nanomedicine, is part of current programs in Dr. Liang's lab.

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FOOTNOTES

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