11 Inorganic Nanoparticles for Biomedical Applications

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Abstract   Polymer, lipid, metal, semiconductor, and hybrid composite nanoparticles with dimensions < 100 nm, have been developed extensively for potential biomedical applications like drug delivery systems, molecular sensing devices, and diagnostic imaging. In this overview, only inorganic nanoparticles for drug delivery will be addressed. Inorganic nanoparticles exhibit magnetic, electrical and optical properties that differed from their bulk counterparts. These physical properties could be tailored by controlling the size, shape, surface, and domain interactions in the nanoparticles. The incorporation of the unique properties of nanoparticles has expanded alternative platforms for drug delivery. The drug delivery systems highlighted in this overview include unguided, magnetically-guided, and optically-triggered delivery systems. These delivery systems are developed to enable improved localization and control of the drug's sphere of influence. This would potentially allow for more efficient therapy with lower dosages and reduced adverse side effects.

11.1   Introduction

Nanoparticles have driven the development of various biomedical applications, including drug delivery systems, diagnostic imaging, and molecular sensing devices (Jaspreet et al., 2005; West and Halas, 2000, 2003; Prasad, 2004; Holm et al., 2002). In this overview, only inorganic nanoparticles for drug delivery are addressed. Sustained release and targeted drug delivery systems are designed to optimize therapeutic efficiency of drugs and localize the drug's sphere of influence to regions of interest (Jaspreet et al., 2005; Brannon-Peppas and Blanchette, 2004;

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Kost and Langer, 2001). The optimal drug delivery system must not be removed too rapidly from either the systemic circulatory system or region of interest, and maintain a minimal drug leakage away from target site. Drugs released from the carriers should remain functionally active, and unloaded drug carriers should subsequently be cleared from systemic circulation (Petrak, 2006).

The characteristic dimensions of nanoparticles in the length scale of < 100 nm have driven interests for its applications in intravenous delivery, pulmonary delivery, and intracellular delivery. Using nanoparticles, an improved efficiency for pulmonary delivery was achieved (Chavanpatil et al., 2006; Hughes, 2005). For applications of nanoparticles in cancer therapy, liposomes and polymeric drug carriers of $\leqslant$ 100 nm were reported to show increased permeability and localization at tumor sites. This was attributed to reduced diffusive barrier for nanoparticles given that gap junctions of the tumor vasculature were estimated to be ~100 – 600 nm (Allen, 2002; Panyam and Labhasetwar, 2003). Besides particle size, surface properties of nanoparticles would influence cellular uptake and distribution of nanoparticles (Moghimi et al., 2001). Nanoparticles with a more hydrophobic surface are taken up by cells to a greater extent through both endocytosis and phagocytosis than those with a hydrophilic surface.

Surface modification of nanoparticles is often required to improve its stability, compatibility, and functionality. Surface characteristics of nanoparticles have been engineered using surfactants that served as molecular linkers and improved particle stability (Kossovsky et al., 1994; Love et al., 2005; Caruso, 2002; Chan, 2006). Surfactants would reduce the surface energy of nanoparticles and enhance its stability by acting as a barrier to agglomeration through either steric hindrance or repulsive electrostatic forces. Functional groups on surfactants have enabled the coupling of nanoparticles with biomolecules such as drugs or antibodies (see Fig. 11.1). Subsequently, the surface functionalized nanoparticles would be able to serve as drug carriers, with potential for specific localization if particles were also modified with antibodies.
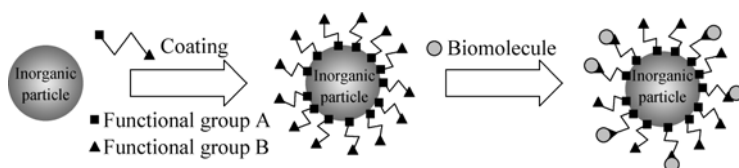

Figure 11.1   Schematic of surface functionalization of inorganic nanoparticles

Ceramics such as calcium phosphates (e.g. hydroxyapatite), silica, and titania are known to be biocompatible (Jin and Ye, 2007). The use of inorganic ceramic nanoparticles offers higher thermal and chemical stability than polymeric nanoparticles. Thus, encapsulation of drugs within ceramic particles would give better protection of labile agents against denaturation (Hasirci et al., 2006). In addition, with the increasing percentage of surface atoms and increasing separation

between energy states (i.e. more discrete energy levels) with decreasing size, inorganic nanoparticles exhibit unique magnetic, electrical and optical properties that differ from its bulk counterparts (Kittel, 2005; Whitesides, 2005; Pitkethly, 2004). Properties of nanoparticles could be tailored by controlling the size, crystal structure, shape, surface, and domain interactions in nanoparticles. For instance, Au nanoparticles of $\leqslant 5$ nm and bulk Au did not exhibit any absorption within the visible range, while Au nanoparticles with diameters of $5 - 100$ nm showed a distinct size-dependent absorption band at $520 - 570$ nm (Kreibig and Vollmer, 1995; Link and El-Sayed, 1999, 2000). In addition, optical properties of metallic nanoparticles could be modified by using different surfactants to tailor optical properties (Persson, 1993; Linnert et al., 1993; Hilger et al., 2000; Salgueiriño-Maceira et al., 2003; Pinchuk et al., 2004). Therefore, physical properties of inorganic nanoparticles could be exploited to enable local release at regions of interest with tunable sensitivity and responsiveness. Drug-loaded magnetic nanoparticles could be guided under an applied magnetic field to a specific area for subsequent release. Similarly, localized release could be achieved using light to trigger drug release from optically-active drug nanocarriers.

The drug delivery systems highlighted in this overview are unguided, magnetically-guided and optically-triggered delivery systems. An introduction to the different approaches adopted by each drug delivery system will be described. An overview of different chemical synthetic and surface functionalization methods for the different carriers are also presented. Besides some commonly used ceramic nanoparticles like hydroxyapatite, considering that most inorganic nanoparticles are non-resorbable and non-degradable with unknown in vivo clearance, significant nanoparticle accumulation within the systemic circulation may occur (Singh et al., 2006). With the increasing dominance of size and surface effects at the nanoscale, toxicity assessments of conventional bulk materials (U.S. Food and Drug Administration, 2000) and microparticles (Foster et al., 2001) may not be applicable. Thus, it would be crucial to evaluate cytotoxic effects of nanoparticles (Hood, 2004; Kagan et al., 2005; Barnard, 2006; Sayes et al., 2004; Kirchner et al., 2005). Cytotoxic properties of non-degradable magnetic and optically-active inorganic nanoparticles would be mentioned in this chapter.

11.2 Unguided Drug Delivery Systems

Ceramic nanoparticles have mostly been developed for sustained drug release systems to enhance delivery efficiency, reduce undesired systemic effects and improved convenience to patients. The most popular ceramic materials used in clinical applications are silica and calcium phosphate (e.g. hydroxyapatite). Besides non-viral gene delivery, their most significant contribution was found as substrates for tissue engineering, bone regeneration and bone repair (Vallet-Regi, 2006). In

particular, calcium-based ceramic materials have an advantage over other polymeric materials for bone repair due to its better bonding to living bone and its ability to catalyze bone growth due to increased nucleation of apatite. For instance, hydroxyapatite is a ceramic similar to the mineral part of bone.

Biomolecules and therapeutic agents were combined with ceramic particles to develop materials that functioned as controlled release systems (Vallet-Regi, 2006; Gadre and Gouma, 2006). Performance of biodoped ceramics were governed by the chemical activity and functionality of attached biomolecules. Adsorption properties of ceramic materials are governed partly by pore size, matrix structure, and surface functional groups. Surface functional groups could be further modified using different chemical species to expand its ability to attach different biomolecules. The chemical activity of biomolecules would be influenced by interfacial interactions between biomolecules and ceramic matrix. An ideal biodoped ceramic would exhibit a good long term stability under potentially adverse conditions, high loading density for biomolecules that remains bioactive, and resistant to leaching of biomolecules (Vallet-Regi, 2006). Biodoped ceramic materials have been used either as a porous solid piece (e.g. implants) or in injectable form for development of non-invasive surgical applications (Vallet-Regi, 2006; Ben-Nissan, 2004; Hou et al., 2004; Temenoff and Mikos, 2000).

11.2.1   Chemical Synthesis of Ceramic Nanomaterials

Main chemical synthetic methods for ceramics include sol-gel processing and precipitation. The sol-gel technique typically involved hydrolysis of metal alkoxide, M(OR)x precursors in the presence of alcohol as a co-solvent to form metal hydroxyls (Klein, 1996; Livage et al., 1998; Vioux, 1997; Oskam, 2006; Brinker and Sherrer, 1990; Niederberger and Garnweitner, 2006). This was followed by condensation of metal hydroxyl groups, where either water or alcohol as by product. Some processing parameters commonly used to control nucleation and growth kinetics were chain length of metal alkoxide precursors, surfactants, solvent (molecular weight of alcohol), temperature, pH, and mechanical agitation. As condensation continues, larger aggregates were formed and viscosity of sol increases resulting in subsequent formation of a gel. Capillary forces during solvent removal from the pores would lead to gel shrinkage. Using the Stöber method developed from sol-gel principles, silica particles with a range of different sizes was prepared (Green et al., 2003). Another common method, particularly for hydroxyapatite, is precipitation of metallic salt (calcium nitrates) with a base (ammonium phosphate) (Ahn et al., 2001; Kumta et al, 2005). The nucleation and growth kinetics were primarily governed by the pH of the starting solution (Ahn et al., 2001; Kumta et al., 2005). The degree of crystallization and prevention of growth of undesired secondary phases were controlled by using different aging temperatures.

11.2.2   Functionalization of Ceramic Nanomaterials

Silica nanoparticles without surface modification did not seem to condense and deliver DNA (Luo and Saltzman, 2006). For DNA delivery using nanoparticles, the extended long chain DNA molecules were condensed to reduce its occupied spatial volume. The presence of surface amino groups on silica nanoparticles upon modification with aminosilanes, would enable it to tightly bind with plasmid DNA and serve as a gene delivery carrier. Using silica nanoparticles as a gene delivery carrier would prevent DNA from being degraded by environmental enzymes. In addition, it was reported that DNA-loaded silica nanoparticles showed an enhanced cellular uptake when compared to other commercial DNA transfection vectors available. Silica nanoparticles were found to enhance the transfer efficiency of commercially available transfection vectors by a factor of 1 – 7 (Luo, 2005; Xu et al., 2006). Another commonly used non-viral gene delivery carrier is calcium phosphate (apatite, hydroxyapatite, and carbonated apatite) based materials. Calcium phosphate materials are suitable candidates as a gene delivery carrier due to its biocompatibility, biodegradability and known adsorptive capacity of DNA on bare calcium phosphate. Calcium phosphate gene carriers were prepared through the co-precipiation of calcium phosphate particles with DNA (Luo, 2005; Olton et al., 2007; Zhu et al., 2004). However, lower levels of gene expression in comparison to viral approaches were observed. This was associated with difficulties associated with endosomal escape, insufficient protection of DNA from nuclease degradation, and inefficient nuclear uptake.

Biomolecules were often encapsulated using a modified sol gel process with addition of amino acids, sugars, or cytoprotecting agents like glycerol or other polymer additives like polyethylene glycol (Avnir et al., 2006; Coradin and Livage, 2007). Protein encapsulation within rigid ceramic pores of similar dimensions offers protection against other denaturing forces in the presence of an organic solvent or extreme pH. Porous blocks of calcium hydroxyapatite and tricalcium phosphate were evaluated as sustained release system fro anticancer drugs, cisplatin, and methotrexate (Uchida et al., 1992; Itokazu et al., 1998). In addition, hybrids of ceramic and polymeric materials mixed with therapeutic agents (e.g. tissue growth factors or small molecule drugs) are often used. Polymeric-ceramic hybrids offer a combination of properties that were unique to either polymeric or ceramic materials alone. Properties of such polymeric-ceramic hybrids would depend on the percentage of each constitutive component present. A sustained release strategy developed was the use of a polymer (poly(lactic-*co*-glycolic acid)) with acidic degradation products to control the dissolution of a basic inorganic component (apatite) on which a therapeutic agent (e.g. bone morphogenetic proteins) were adsorbed (Yong et al., 2004; Yong, 2005). The release profile could be altered by changing variables that affect polymer degradation (type, molecular,and composition) and/or apatite dissolution (loading and particle size).

11.3   Magnetically-Guided Drug Delivery Systems

11.3.1   Magnetic Guiding

Magnetically-responsive delivery systems introduced into the systemic circulation are directed to regions of interest using an applied magnetic field. The external magnetic field of ~0.8 – 1.7 T, may be a magnet or an array of magnets placed near a lesion or tumor, either externally placed or implanted. (Hayden and Häfeli, 2006; Gould, 2006; Alexiou et al., 2006; Jurgons et al., 2006). Therapeutic agents were subsequently released using either another trigger such as ultrasonic waves or an alternating magnetic field (Kost and Langer, 2001; Tirelli, 2006; Frimpong et al., 2007). Magnetically modulated drug delivery systems prepared using large magnetic particles embedded in a polymer matrix, were shown to enhance drug release rates upon application of an oscillating magnetic field. Drug release rates were shown to be dependent on the characteristics of magnetic field (e.g. field strength, and amplitude) and polymer properties (e.g. rigidity of polymer matrix). When the holding magnets were removed, the particles would either redistribute into the blood supply to be eventually cleared by the reticuloendothelial system or remain within the region of interest to be cleared by extravasation.

Recent studies demonstrated successful localization of doxorubicin-loaded 200 nm Fe@C particles, and 80 nm to 2 µm silica-coated $Fe_3O_4/\gamma\text{-}Fe_2O_3$ particles using implanted Au-plated permanent magnets. The magnetic implants were Au-plated to improve chemical stability and biocompatibility. Though high particle concentrations were found in the liver, magnetic carriers were drawn to the left kidney close to an implanted magnet whereas no particles were observed in right kidneys of the rabbits tested. Similar studies using externally placed magnets showed successful localization of magnetic particles at the peritoneal cavity of mice. The efficacy and potential for pulmonary embolism of magnetically-guided drug delivery systems would be governed by physiological parameters, temporal localization kinetics, and microcirculatory flow (Lübbe et al., 1999).

11.3.2   Chemical Synthesis and Properties of Magnetic Nanostructures

Iron-based magnetic nanomaterials, particularly magnetite (Fe_3O_4) or maghemite ($\gamma\text{-}Fe_2O_3$) due to its better chemical stability and lower toxicity, are most commonly used compared to alternative cobalt- and nickel- based nanomaterials (Qiang et al., 2006). Magnetic nanoparticles have been prepared using several chemical methods to control nucleation and growth rates. The size, morphology, and

composition of particles synthesized were governed by the nucleation and growth rates, whereas the size dispersion would depend on decoupling of nucleation and growth rates (Turkevich et al., 1951; Stokes and Evans, 1997; Sheludko, 1996; Davey and Garside, 2000; Huber, 2005). Base precipitation of iron salts from a surfactant-containing aqueous solution at low temperatures was a common chemical method for aqueous synthesis of magnetic nanoparticles (Chatterjee et al., 2003). Another synthetic method is the thermal decomposition of organometallic precursors in high-boiling solvents in the presence of stabilizing ligands, such as oleic acid or oleyl amine. Organometallic-based synthesis often resulted in magnetic nanoparticles with a narrow size distribution ($\sigma < 5\%$) and high degree of crystallinity (see Fig. 11.2) (Lin and Samia, 2006; Park et al., 2004; Behrens et al., 2006; Hyeon, 2003).
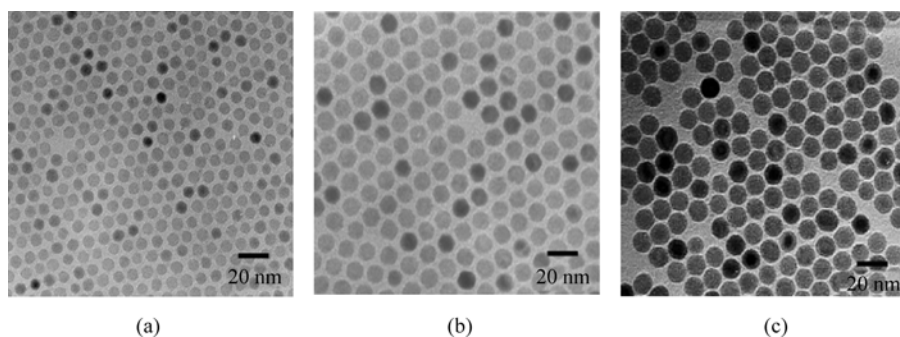

(a)   (b)   (c)

Figure 11.2 (a) 7 nm (b) 11 nm and (c) 13 nm γ-Fe$_2$O$_3$ nanoparticles synthesized using thermal decomposition of organometallic precursors (Hyeon, 2003; Hyeon et al., 2001). Adapted from (Hyeon, 2003). Reprinted with permission from (Hyeon et al., *J. Am. Chem. Soc.* 2001). Copyright (2001) American Chemical Society

The magnetic particles must exhibit high magnetization and superparamagnetic behavior at room temperature for guidance and immobilization to region of interest (Neuberger et al., 2005). Particles with high saturation magnetization would be localized more easily to a region using lower external magnetic fields. As the size decreases, thermal energy may be sufficient to give rise to fluctuations of magnetization directions. Thus, particles below a critical size (e.g. ~10 nm for Fe$_3$O$_4$ or γ-Fe$_2$O$_3$) would be magnetized by an applied magnetic field but retain no permanent magnetism upon removal of the applied field. Particles with this superparamagnetic behavior would have reduced tendency for agglomeration that was driven by magnetic attractive forces. While decreasing particle size would result in the desirable superparamagnetic behavior, a trade-off would be reduced saturation magnetization. Smaller particles with lower saturation magnetization may have less effective localization, while larger particles may have lower cellular uptake and higher chances for embolism.

11.3.3 Functionalization of Magnetic Nanoparticles

Surfaces of magnetic nanoparticles are modified with polymeric, metallic or oxide surface to improve either its stability against agglomeration, bioavailability and biomolecular functionalization (A.K. Gupa and M. Gupa, 2005; Berry and Gurtis, 2003). Particles were often surface functionalized with either an organic, polymeric or inorganic layer using either ligand exchange or encapsulation methods (Hong et al., 2005; Bruce and Sen, 2005; Templeton et al., 2000). Particles were coated with a layer of organic surfactants during chemical synthesis to prevent agglomeration. However, such surfactants might not provide the necessary chemical functionality needed for its eventual applications. Thus, surfactants covering the particles were replaced with other surfactants using various ligand exchange methods. Particles could be encapsulated by a polymeric coating by precipitation of inorganic salts in an aqueous polymer solution (Yu and Chow, 2004; Babes et al., 1999). For instance, Fe_3O_4 or γ-Fe_2O_3 particles were commonly encapsulated with dextran or starch for improved biocompatibility and solubility. It was also demonstrated that after surface modification, an anticancer drug (carboplatin) was bound to polymethyl metacrylic acid coated γ-Fe_2O_3 particles (Fig. 11.3) (Yu and Chow, 2004). Silica-coated particles provide enhanced stability and surface silanol groups for covalent coupling (Bruce and Sen, 2005). After hydrolysis and condensation of organosilanes that were deposited on the particle surface, a silica coating was formed. Characteristics of the final surface layer depended on reaction variables such as solvent type, temperature, or time, as well as on the catalyst and organosilane concentrations used.
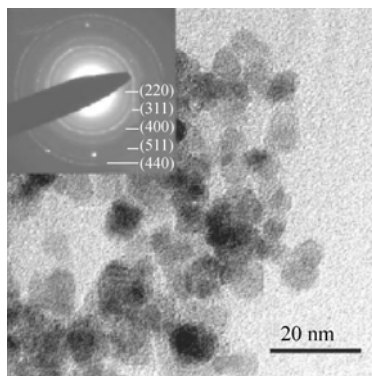

Figure 11.3 Transmission electron micrograph and corresponding electron diffraction pattern of polymethyl acrylic acid coated γ-Fe_2O_3 particles (Yu and Chow, 2004). —Reproduced by permission of The Royal Society of Chemistry

11.3.4 Biocompatibility of Magnetic Nanoparticles for Drug Delivery

It was reported that besides intrinsic size effects, the surface coating had an important role in the cytotoxicity of oleic acid coated nickel ferrite nanoparticles (Yin et al., 2005). It was found that particle size of uncoated nickel ferrite was not a significant factor on cytotoxicity when there were no 'toxic' functional groups on particle surface. In contrast, oleic acid-coated nickel ferrite particles of ~150 nm and ~10 nm were cytotoxic. If oleic acid molecules were present as monomer, they were not cytotoxic. However, if they developed micelles or coated on the ferrite particles, i.e. when their functional groups were spatially aligned, cytotoxicity was observed. Larger particles had a larger cytotoxic effect than smaller particles when one or two layers of oleic acid were deposited on particle surface. This could be related to surfactant reactivity and interfacial interaction areas that were dependent on particle size. The difference in surface energies with particle sizes may have affected surfactant conformation, which may alter the surfactant reactivity. Thus, the same surfactant may behave differently when it interacts with cells. Also, with a larger effective interaction area for a larger particle compared to that for a smaller particle as shown in Fig. 11.4, the larger particle will exert a larger localized stimulus on the cells. It was subsequently suggested in the report that that a single localized stimulus from a larger particle was stronger and more toxic than an equivalent sum of stimuli at different locations exerted by smaller particles.
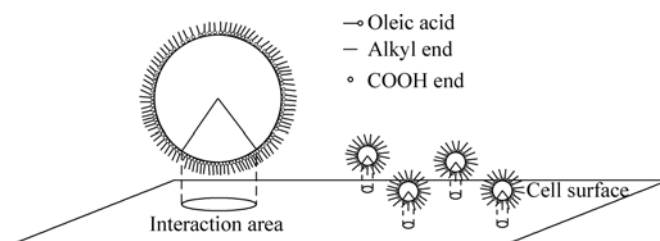

Figure 11.4 Sketch of the various interaction areas of individual particle with one layer oleic acid coating. A single large particle possessed larger interaction areas with more function groups. While, the big number of small particles increased number of interaction points, there were less functional groups at each interaction point (Yin et al., 2005). —Reprinted from H. Yin, H.P. Too and G.M. Chow. The effects of particle size and surface coating on the cytotoxicity of nickel ferrite. *Biomaterials*, 26: 5818 − 5826, Copyright (2005), with permission from Elsevier

11.4 Optically-Triggered Drug Delivery Systems

Near infrared (NIR) light ($\lambda = 650 - 1000$ nm), with its deep penetration in living tissues shown in and high signal-to-background ratio, has been exploited for biomedical imaging, photoablation and photodynamic therapy (Frangioni, 2003;

Sato et al., 2001; Dolmans et al., 2003). The extent of NIR light propagation would be governed by absorption and scattering properties of tissues (Waynant et al., 2001; Niemz, 2002; Vogel and Venugopalan, 2003). Major tissue absorbers of NIR light are hemoglobin, melanin, and water, while composition, size, and morphology of tissue components control the light scattering. The absorption and scattering properties would influence volumetric energy distribution induced by laser irradiation, and set the boundaries for localized NIR-activated drug release systems. NIR light was reported to travel through 10 cm of breast tissue and 4 cm of skull tissue using microwatt sources (Weissleder, 2001). A targeted drug delivery system that incorporated NIR-sensitive nanoparticles and tissue penetrative NIR light to trigger drug release was developed (Ren and Chow, 2003; Chow et al., 2006; Tan, 2006). This could potentially improve chemotherapy treatment by minimizing deleterious side effects and allowing minimally invasive treatment of surgically inoperable tumors.

11.4.1   Chemical Synthesis and Properties of NIR-Sensitive Nanoparticles

Metallic nanoparticles and metallic nanoshells with size-dependent optical properties, particularly chemically stable Au nanoparticles, have been utilized as molecular sensors (West and Halas, 2003; Prasad, 2004; Holm et al., 2002). As sensors, binding of molecules would give rise to either fluorescence enhancement or plasmon resonance shifts. NIR-sensitive metallic nanoshells (Oldenburg et al., 1998). with size and shell thickness dependent properties were investigated for applications in imaging (Loo et al., 2005), hyperthermia (Hirsch et al., 2003), temperature-responsive delivery systems (Sershen and West, 2006), and immunoassays (Hirsch et al., 2003, 2006). For the NIR-activated drug delivery system, the NIR-sensitive nanoparticles were synthesized by reduction of $HAuCl_4$ with Na_2S (Ren and Chow, 2003; Chow et al., 2006; Zhou et al., 1994; Averitt et al., 1997). These as-synthesized nanoparticles were chemically stable and exhibited two absorption bands at ~530 nm and in the NIR region of $650 - 1100$ nm. The as-synthesized nanoparticles were composites of crystalline Au and amorphous Au_2S (Tan, 2006; Tan et al., Submitted). The NIR absorption was unique to as-synthesized nanoparticles, and was absent in either Au or Au_2S nanoparticles. There was no evidence that NIR absorption properties were related to a core-shell structure as suggested in earlier work in the literature. Therefore, NIR absorption was likely due to interfacial effects on particle polarization from introduction of amorphous Au_2S in a predominantly crystalline Au matrix (Tan, 2006; Tan et al., Submitted). The effects of concentration ratios for precursors used in the chemical synthesis of nanoparticles were correlated with the resultant NIR properties. Consequently, by varying concentration ratios of precursors, the optical properties of as-synthesized nanoparticles were tailored (Chow et al., 2006; Tan, 2006). This ability to tailor

optical properties would advance potential use of as-synthesized nanoparticles for optically-activated drug delivery systems or other biomedical applications.

11.4.2   Functionalization of NIR-Sensitive Nanoparticles

Functionalization of NIR-sensitive Au-Au$_2$S nanoparticles with surfactants has facilitated the loading of anticancer drugs as shown in Fig. 11.5 (Chow et al., 2006; Tan, 2006). Surfactants of different hydrocarbon chain lengths were used to modify the nanoparticles, and subsequently altered interfacial interactions between nanoparticles and surfactants. The loading of anticancer drugs governed by surfactant interfacial interactions was correlated to the surfactant chain length. In addition, inorganic-organic interfacial interactions between nanoparticles and surfactants may be used to manipulate the optical properties of NIR sensitive drug delivery system. Drug release was triggered upon NIR laser irradiation using a Nd:YAG pulse laser at $\lambda = 1064$ nm (Ren and Chow, 2003). The structural and microstructural changes of Au-Au$_2$S nanoparticles upon NIR laser irradiation were studied. Insights to NIR triggered drug release process were elucidated from the estimated magnitude of thermal effects and structural and microstructural changes induced by NIR irradiation (Tan, 2006).
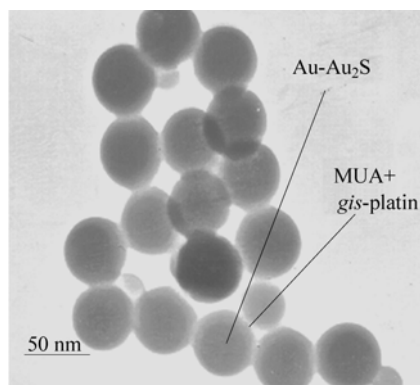

Figure 11.5   Cisplatin-loaded surface modified Au-Au$_2$S nanoparticles (Ren and Chow, 2003).—Reprinted from L. Ren and G.M. Chow. Synthesis of NIR-sensitive Au-Au$_2$S nanocolloids for drug delivery. *Materials Science and Engineering* C, 23: 113 – 116, Copyright (2003), with permission from Elsevier

11.4.3   Biocompatibility of NIR-Sensitive Nanoparticles for Drug Delivery

The in vitro cytotoxicity of the NIR-sensitive Au-Au$_2$S drug delivery system was assessed using breast cancer cells for its potential clinical application (Tan, 2006).

The in vitro cytotoxicity of surfactant-modified nanoparticles and drug-loaded-surfactant-modified nanoparticles were investigated. (Tan, 2006) It was found that the in vitro toxicity of drug-loaded-surfactant-modified nanoparticles depended on the surfactant used for drug adsorption. The in vitro cytotoxic effects of released drugs in the supernatant fraction collected after NIR irradiation of drug-loaded-surfactant-modified nanoparticles were evaluated. It was found that the released drug was chemically modified with increased toxicity. In addition, transmission electron microscopy (TEM) micrographs indicated that both Au-Au$_2$S nanoparticles and cisplatin-loaded Au-Au$_2$S nanoparticles were found associated with the plasma membrane or in small vesicles within cells. Studies on in vitro carcinogenicity of nanoparticles using a medium-term (25 days) NIH/3T3 cells transformation test was also conducted (Ren et al., submitted). Examination using light microscopy of the cells exposed to the Au-Au$_2$S NPs and cisplatin-loaded Au-Au$_2$S nanoparticles revealed morphological alterations when compared to that of control cells. However, no difference in cell morphology among cells exposed to cisplatin-loaded Au-Au$_2$S nanoparticles was observed. This indicated that cisplatin-loaded Au-Au$_2$S nanoparticles did not cause carcinogenicity in vitro below a maximum recommended dosage in the given system.

Similar in vivo biodistribution profiles (Fig. 11.6) of nanoparticles using both intra-tumor and tail vein injection administration routes were observed (Huang et al., submitted). Most particles accumulated in the reticulo-endothelial system, mainly liver and spleen. Small amounts of Au-Au$_2$S nanoparticles were found in the lung, probably due to embolism of agglomerated nanoparticles in lung capillaries. While the mass of Au-Au$_2$S NPs in the kidneys increased to 0.95 μg/g within 7 days, no Au-Au$_2$S NPs were deposited in other organs like brain, heart, muscle, bone, intestine, and blood. Using the tail vein injection for particle administration resulted in reduced concentration in the biodistribution profile
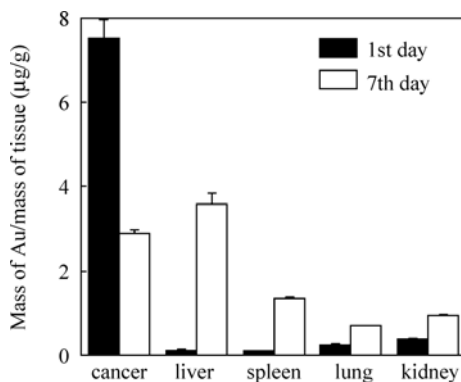

Figure 11.6   Biodistribution of Au-Au$_2$S NPs in KM mice treated by intra-tumor injection at different time points (Huang et al., submitted)

compared to that of using the intra-tumor route. This was due to the enhanced penetration and retention effect of tumors for particles of $50 - 100$ nm. This suggested that intra- tumor injection may improve local tumor response and minimize any systemic side effects.

In summary, preliminary findings from the short and long-term in vitro suggested that cisplatin-loaded Au-Au$_2$S nanoparticles were non-toxic below a maximum recommended dosage. It remains premature to draw definitive conclusions about the toxicities, if any, of Au-Au$_2$S nanoparticles. Further work is required to evaluate if the unique physicochemical properties of Au-Au$_2$S nanoparticles would introduce other injurious mechanisms and pathological lesions.

11.5   Summary

The incorporation of the unique properties (chemical stability, magnetic and optical) of inorganic nanoparticles has expanded alternative platforms for drug delivery. These drug delivery systems were developed to enable improved localization and control of the drug's sphere of influence. This would potentially allow for more efficient therapy with lower dosages and reduced adverse side effects. A multidisciplinary approach would be needed for advancement of the delivery systems to its eventual applications. Optimization of chemical synthetic and functionalization methods to improve quality (distribution, loading capacities) of drug carrier would facilitate developmental progress of drug delivery systems. Further scientific development on understanding of release mechanisms for better engineering control of these drug delivery systems would be required. More detailed in vitro and in vivo work on the nanoparticle-cellular interfacial interactions and its implications on safety of inorganic nanoparticles would also be critical.

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