Recent Advances in Inorganic Nanoparticle-Based Drug Delivery Systems

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Abstract: Drug delivery systems, designed to enhance drug efficacy and reduce their adverse effects, have evolved accompanied by the development of novel materials. Nanotechnology is an emerging scientific area that has created a variety of intriguing inorganic nanoparticles. In this review, we focus on the feasibility of inorganic nanoparticles, including iron oxide nanoparticles, gold nanoparticles, fullerenes and carbon nanohorns, as drug carriers, and summarize recent advances in this field.

Key Words: Nanomedicine, drug delivery, gene delivery, drug carrier, iron oxide nanoparticle, gold nanoparticle, fullerene, carbon nanohorn.

INTRODUCTION

Over the past few years, there has been great interest in applying nanotechnology to the field of medicine, a combination that has recently been referred to as "nanomedicine". According to the National Institutes of Health Roadmap Initiative in the United States, nanomedicine encompasses specific intervention with devices and structures operating within the range of 1-100 nm, which is the scale of most components of biomolecular complexes [1]. Nanomedicine is expected to lead to the development of tools for drug delivery, imaging and sensing. Research into the delivery and targeting of pharmaceutical, therapeutic, and diagnostic agents *via* intravenous and interstitial routes of administration, using nanoparticles, is at the forefront of nanomedicine projects. Indeed, increased knowledge in the field of nanotechnology and nanofabrication has had an immediate impact on the field of drug delivery.

Drug delivery systems are designed to improve the pharmacological and therapeutic properties of drugs administered *in vivo*, and include particulate carriers that can function as drug reservoirs [2-5]. Drug-carrier complexes achieve controlled release of drugs and/or targeting of them to desired sites, resulting in alteration of the pharmacokinetics and biodistribution of the drugs [6]. In this sense, nanoparticles are intrinsically advantageous over conventional particles. The pharmacokinetics of particulate drug carriers is largely affected by splenic filtration, which occurs at the interendothelical cell slits (< 200 nm) in the walls of venous sinuses [7, 8]. Furthermore, vesicles larger than 100 nm must be designed to prevent surface opsonization processes, which lead to phagocytic uptake of the particles in liver. Taken together, nanoparticles can be a long-circulatory drug carrier. Based on enhanced permeability and retention (EPR) effects [9, 10], this kind of drug carriers is useful for cancer chemotherapy.

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Among the drug carriers reported so far, liposomes [11, 12] and lipid microspheres [13] have come onto the market. Polymer micelles are currently in clinical trials [14]. In addition, polymeric nanoparticles have received increasing attention [15]. All of these are organic nanoparticles consisting of lipids and/or synthetic polymers. Compared with the rapid progress in the development of drug delivery systems using organic nanoparticles, relatively less progress has been made in the development of inorganic nanoparticle-based drug delivery systems.

Recent progress in the field of nanotechnology and nanofabrication has led to the production of various inorganic nanoparticles as attractive vehicles for drug delivery. In this review we will feature inorganic nanoparticles, including iron nanoparticles, gold nanoparticles, fullerenes and carbon nanohorns (Table **1**). There are several advantages of these inorganic nanoparticles as drug carriers. First, they are easy to prepare with a defined size. More interestingly, they often exhibit multiple functions useful in medicine, for example as exothermic reactors and contrast agents, whereas organic nanoparticles such as liposomes and microspheres serve only as drug reservoirs. The emerging roles of these inorganic nanoparticles in drug delivery systems are the focus of this review.

IRON OXIDE NANOPARTICLES

Iron oxide nanoparticles of 50-100 nm in diameter were first developed as contrast agents for magnetic resonance imaging in the late 1980s [16]. These nanoparticles were solubilized in water by dextran surface coating. When intravenously administered to hepatocarcinoma patients, they rapidly accumulated within macrophages in the liver, such as Küpffer cells; this enabled cancer tissues to be negatively visualized, because cancer tissues contain fewer macrophages [16]. On the contrary, in order to evade such a rapid accumulation in the liver and spleen, and prolong their half-life in circulation, much smaller iron oxide nanoparticles with a diameter of ~ 5 nm, designated ultrasmall superparamagnetic iron oxide (USPIO) particles, were produced [17]. Interestingly, USPIOs intravenously administered to rats accumulated in the lymph nodes in iliac, celiac, paraaortic, mesenteric, and mediastinal regions. The total concentration

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Table 1. Pre-Clinical Studies on Inorganic Nanoparticles Outlined in this Review

Nanoparticle	Payload	Pre-Clinical Model	Outcome	Mechanism of Action	Ref.
Iron oxide					
	None	Renal cell carcinoma in mice	Remission	Hyperthermia	[24]
	Mitoxantrone	Squamous cell carcinoma in rabbits	Remission	Magnetic drug targeting	[27]
Gold					
	Plasmid DNA	Normal mice	Electric pulse-assisted gene expression	Systemic gene delivery	[49]
	TNF-α	Colon or mammary carcinoma in mice	Regression	Imaging/drug delivery hyperthermia	[53,54]
	None	Colon carcinoma in mice	Resorption	Photothermal therapy	[60]
Fullerene					
	Substituents (for anti-HIV therapy)	Normal rat	Rapid clearance from plasma		[66]
Carbon nanohorn					
	Doxorubicin, Cisplatin	In progress			

of USPIOs in the lymph nodes was 3.62 ± 0.64%, while that in liver and spleen was 6.32 ± 0.22 and 7.12 ± 0.57%, respectively [17]. Furthermore, it was reported that USPIO could detect axillary lymph nodes in patients with breast cancer [18] and mediastinal lymph nodes in patients with primary lung cancer [19].

Magnetite nanoparticles of around 10 nm in diameter are attractive compounds with which to achieve hyperthermia and active targeting for cancer therapy [20, 21]. Since the heat generation activity of each single magnetite nanoparticle is very low, the magnetite nanoparticles have been encapsulated by cationic liposomes [22] or immunoliposomes [23, 24] to enhance heat generation. Immunoliposomes intravenously injected into a mouse model of human renal carcinoma were reported to accumulate in tumor tissue overexpressing the antigen with an efficiency of 50%, and to show magnetic field irradiation-dependent anti-tumor activity [24].

The feasibility of magnetite nanoparticles for active drug targeting was reported more than 20 years ago [25]. In that report, magnetite nanoparticles were mixed with cottonseed oil, human serum albumin and the anticancer drug doxorubicin to prepare magnetic microspheres with an average diameter of 1 μm. The magnetic microparticles could be targeted to tumor sites in rats under magnetic field irradiation. Since there have been concerns about the long-term deposition of aggregated magnetic particles *in vivo* and the need for strong magnets with constant field gradients, *in vivo* application of magnetite nanoparticles needs further improvement [26].

Several groups have been trying to overcome these problems of magnetite-containing drug carriers. Since the physical properties of the emulsified magnetic particles are problematic and not suitable as drug carriers, as mentioned above, Lübbe and coworkers produced magnetite nanoparticles that could adsorb drugs directly [27-29]. The diameter of these nanoparticles is <200 nm, and the magnetite core was coated with starch polymers to stabilize the magnetic particles and allow chemoabsorptive binding of an anticancer drug, mitoxantrone, through ionic interactions between the phosphate groups of the polymers and the amine groups of mitoxantrone (Fig. (**1a**)) [27]. These magnetite nanoparticles complexed with mitoxantrone could release all of the mitoxantrone they were carrying within 60 min. When injected intraarterially (femoral artery) near a tumor site in rabbits, under magnetic field irradiation (1.7 Tesla), complete and permanent remission of the tumor was obtained with no sign of toxicity. Furthermore, the intratumoral accumulation of the magnetic nanoparticle was visualized both histologically and by magnetic resonance imaging. In the case of intravenous injection through the ear vein, however, the antitumor activity was largely attenuated. This may indicate that there remains a need to improve the stability of these magnetic particles *in vivo*.

During the last few years, a series of methods for the surface modification of magnetite nanoparticles with polymers have been reported. Conversion of hydroxyl groups on the surface into amine groups using γ-(aminopropyl)trialkoxysilane was reported [30, 31]. The converted amine groups were easily reacted with carboxyl groups at the terminus of poly(ethylene glycol) (PEG) [31] or the aldehyde groups of oxidized dextran [30]. However, these surface-modified magnetite nanoparticles are not suitable to adsorb chemotherapeutic drugs onto their surfaces, because the neu-

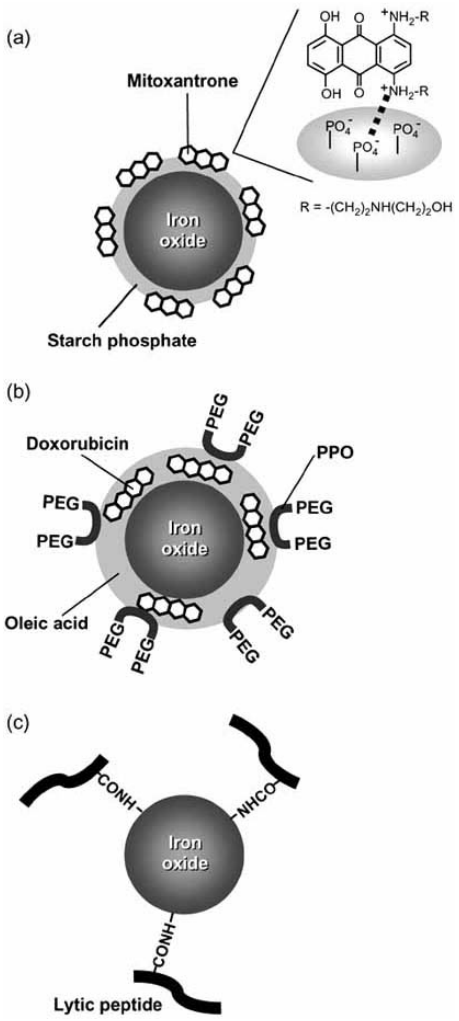


Fig. (1). Schematic drawing of various iron oxide nanoparticle-based drug carriers. (a) Iron oxide nanoparticle coated with starch polymers to adsorb mitoxantrone onto the surface through ionic interactions. (b) Iron oxide nanoparticle coated with oleic acids to adsorb both doxorubicin through PEG-PPO-PEG block co-polymers. (c) Iron oxide nanoparticle covalently modified with lytic peptides through amide linkage.

tral and hydrophilic polymers are poor adsorbents for drugs. By contrast, Jain *et al*. developed a two-step coating procedure to endow magnetite nanoparticles with biocompatibility as well as drug-loading capacity [32]. In the first step, each magnetite nanoparticle was emulsified with oleic acid (OA), which was expected to confer dual functions on the nanoparticles. These OA-coated magnetite nanoparticles were further coated with a PEG-polypropylene oxide (PPO)-PEG block co-polymer. It is expected that the hydrophobic segments of PPO anchor at the surface of the OA shell around the magnetite nanoparticle, and that the hydrophilic segments of PEG extend into the aqueous phase (Fig (**1b**)). Importantly, the double-coated nanoparticles were able to entrap an anticancer drug, doxorubicin (DXR), in the OA layer, at 8.2 wt % efficiency, and slowly release about 62% of the bound DXR over a period of one week. Their antiproliferative effect on human breast and prostate cancer cells was also confirmed *in vitro*.

The covalent attachment of drugs to magnetite nanoparticles is an alternative approach. Tong *et al*. reported modification of naked magnetite nanoparticles prepared by coprecipitation of ferrous and ferric ions in an ammonia solution with bovine serum albumin, by using carbodiimide [33]. Using this method, Kumar *et al*. prepared lytic peptide-bound magnetite nanoparticles (Fig. (**1c**)) [34]. The resulting nanoparticles retained superparamagnetism as well as lysis activity toward human breast cancer cells *in vitro*, indicating that lytic peptides also function as hydrophilic and biocompatible polymers. In summary, magnetite nanoparticles offer attractive drug carriers due to their abilities to perform hyperthermia and targeted delivery.

GOLD NANOPARTICLES

In the mid-19th century, colloidal gold nanoparticles were prepared through the chemical reaction of gold chloride with sodium citrate [35]. Gold compounds have been observed to decrease cellular proliferation and to reduce antibody and cytokine release [36]. It is of note that several gold compounds, like Auranofin, have been clinically approved and used for the treatment of human rheumatoid arthritis [37, 38].

There are a number of good reasons to use gold nanoparticles as carriers for drug delivery. First of all, gold nanoparticles are relatively easy to synthesize with a defined size [39], and they have low cytotoxicity [40]. Surface modification with multiple polymers and ligands can be also achieved in a one-pot synthesis because the gold surface reacts with thiol-groups to form covalent bonds [39]. It should also be noted that gold nanoparticles can be accurately quantified at a sensitivity as low as 0.001 ppm, by instrumental neutron activation analysis [41].

Like magnetite nanoparticles, gold nanoparticles work as efficient photothermal agents in therapeutic applications [42]. The absorption maximum of gold nanoparticles is tunable based on their size and shape. For example, in order to achieve cancer cell-specific hyperthermia, gold nanoparticles could be conjugated with an anti-epidermal growth factor receptor antibody, since cancer cells often overexpress epidermal growth factor receptor on their surfaces to enhance their growth [43]. When the antibody-conjugated gold nanoparticles were incubated with human cancer cells overexpressing epidermal growth factor receptor, and then cells were irradiated with an argon laser at 514 nm, cell death occurred within the laser spot. No cell death was observed for benign or normal cells by laser irradiation. Unlike magnetite nanoparticles, gold nanoparticles were reported to show antiangiogenic properties through neutralizing angiogenic cytokines such as vascular endothelial growth factor [44].

Gold nanoparticles have received considerable attention as DNA carriers using “gene gun” technology [45]. DNA-coated gold nanoparticles can be transferred into almost any type of cell by the pressure of a compressed gas such as He or N_2 [46-48]. In addition, recent reports have shown that gold nanoparticles intrinsically have DNA-delivering capacity. In these reports, gold nanoparticles were reacted with

various hetero-bifunctional molecules, such as 2-aminoethanethiol [49], *N,N,N*-trimethyl(11-mercaptoundecyl)ammonium [50], or thiol-modified polyethyleneimine [51], to introduce amine groups onto their surfaces (Fig. (**2a**)). The modified gold nanoparticles bound to DNA by ionic interactions. Incubating the resulting complexes with cultured cells was sufficient to induce gene expression in mammalian cells. More importantly, the transfection efficiencies of the DNA-gold nanoparticle complexes were several fold higher than those of DNA-polyethyleneimine complexes used as standard transfection reagents [50, 51]. Gold nanoparticles prepared by Kawano *et al*. are likely to be applicable even to *in vivo* gene transfer after surface modification with PEG-SH for stabilization [49]. When PEG-modified gold nanoparticles complexed with plasmid DNA were intravenously injected into mice, 5% of the DNA was detected in blood at 5 min after injection, and 20% of the injected gold was detected 120 min after injection, suggesting stable blood circulation of these complexes. In addition, gene expression can be controlled after the intravenous injection of PEG-gold nanoparticles/DNA complexes, by local delivery of electric pulses in the specific area of the organ. Induction of proinflammatory cytokines was shown to be at similar levels to that of naked DNA, indicating low immunogenicity of gold nanoparticles. However, caution must be taken since one report showed that hepatocytes exposed to gold nanoparticles were damaged, even in the absence of electric pulses [49].

In addition, Au/Ni nanorods were synthesized for gene transfer by electrochemical deposition of gold nanoparticles into an Al_2O_3 template of 100 nm in a pore diameter [52]. These nanorods were 100 nm in diameter and 200 nm in length, with 100 nm of gold segments and 100 nm of nickel segments. Each segment of the nanorod was selectively reacted with either thiol- or carboxyl-containing molecules to be further modified with transferrin as a targeting ligand, and DNA plasmids. The transfection efficiency of DNA-nanorod complexes with targeting ligands in mammalian cells was higher than that of naked DNA and naïve DNA-nanorod complexes without targeting ligands.

Gold nanoparticles have also been used as therapeutic polypeptide carriers. To date, tumor necrosis factor (TNF) [53, 54] and insulin [55] have been successfully conjugated with gold nanoparticles, which were simply prepared by the reduction of $HAuCl_4$. TNF was allowed to react directly with the surface through the formation of covalent bonds, which were cleavable by treatment with 1 μg/ml dithiothreitol. For insulin binding, the surface was capped with aspartic acid and insulin was adsorbed through hydrogen bonding in a non-covalent manner, because a more rapid release of insulin is favorable. In the case of TNF-immobilized gold nanoparticles, PEG-SH was further introduced to the surface. When these PEG- and TNF-conjugated gold nanoparticles with a mean diameter of ~33 nm were intravenously administered to mice with colon carcinomas, gold nanoparticles accumulated at the tumor site within 3 hours of injection. TNF has been shown to have antitumor activity. However, it evokes toxicity when applied systemically. All mice receiving an injection of native TNF, at a dose of 24 μg per mice, died. However, injection of PEG- and TNF-conjugated gold nanoparticles containing 24 μg of TNF did not cause death.

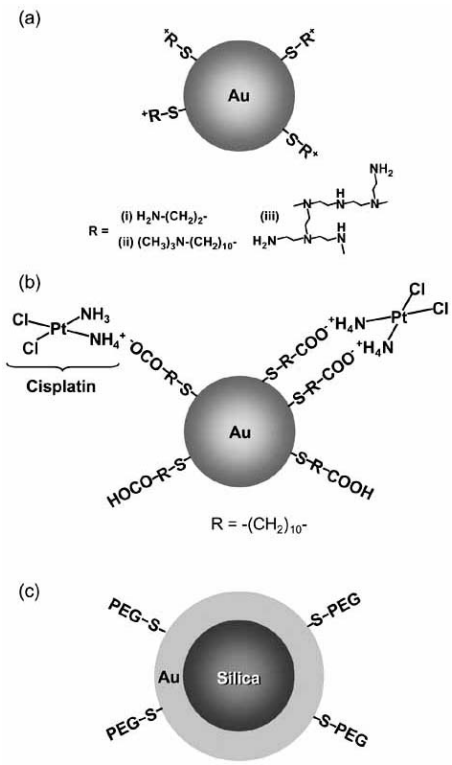


Fig. (2). Schematic drawing of various gold nanoparticle-based drug carriers. (a) Gold nanoparticle covalently modified with (i) 2-aminoethanethiol, (ii) *N,N,N*-trimethyl(11-mercaptoundecyl)ammonium, or (iii) thiol-modified polyethyleneimine for gene delivery. (b) Gold nanoparticle covalently modified with 11-mercaptoundecanoic acids to adsorb cisplatin through ionic interaction. (c) Gold nanoparticle with silica core (gold nanoshell). Gold nanoshell is covalently modified with PEG-SH.

The tumor volume in these mice regressed by more than 90%, indicating that the antitumor action of TNF was effective.

For drugs with low molecular weights, gold nanoparticles, like magnetite nanoparticles, are reported to serve as good carriers. First, Gu *et al*. prepared multivalent antibiotics conjugated to gold nanoparticles by using thiol-containing vancomycin derivatives [56]. These complexes exhibited excellent antibiotic activity against vancomycin-resistant enterococci and *E. coli*. The authors speculated that the multivalency of gold nanoparticles and their binding to substrates on the outer membranes of bacteria played important roles. Secondly, an anticancer drug, cisplatin, was adsorbed onto gold nanoparticles sensitive to near-infrared light, owing to the presence of 11-mercaptoundecanoic acid layers (Fig. (**2b**)) [57]. Based on Fourier transform infrared spectra and ultra violet-visible spectra, cisplatin was suggested to bind the carboxylate groups of 11-mercaptoundecanoic acid *via* ionic interaction. The most intriguing feature of this complex was that about 90% of the cisplatin in these complexes was released into water within 1 min of near-infrared irradiation (1064 nm, 100 mJ/pulse, 7 ns per pulse length, 10

Hz repetition rate), whereas only 40% was released after heating to 40˚C without irradiation.

Gold nanoshells are a new class of near-infrared adsorbing nanoparticle (100-150 nm in dimension) consisting of a spherical dielectric silica core surrounded by a thin layer of gold (Fig. (**2c**)) [58]. An intriguing aspect of this type of gold nanoparticle is its application to photothermal therapy by using near-infrared lasers, which penetrate deeply into tissues. Gold nanoshells were modified with PEG-SH in the same manner as gold nanoparticles [59, 60]. When PEG-gold nanoshells were intravenously administered to mice bearing murine colon carcinomas, and tumors were exposed to near-infrared light (808 nm diode laser, 800 mW at 4 W/cm^2 for 3 min) 6 hours later, complete resorption of the tumor was observed within 10 days [60]. Since gold nanoshell-composite hydrogels were reported to release chemicals and proteins in response to near-infrared irradiation, drugs could be also loaded onto gold nanoshells [61]. Therefore, gold nanoshells could be powerful drug carriers with near-infrared light-assisted dual functions of heat generation and controlled drug release.

In addition, gold porous nanoparticles, designated gold nanocages, were recently synthesized in aqueous solution with $HAuCl_4$, silver nanocubes and poly(vinyl pyrrolidone) [62]. Unlike spherical gold nanoparticles and nanoshells, gold nanocages have hollow nanostructures with porous walls and strong optical resonance peaks in the near-infrared region despite their much smaller dimension (40 nm). These characteristics make gold nanocages suitable for use as contrast agents for optical coherence tomography. They also raise the possibility that gold nanocages may bind much more drug than any other filled nanoparticle, because of their larger surface area [62].

FULLERENE (C_{60})

To date, widespread application of fullerenes and their derivatives in medical fields have been reported. Fullerenes have been used as carriers of anti-human immunodeficiency virus agents [63-66], contrast agents for magnetic resonance imaging [67-69], antioxidants [70, 71], anti-bacterial agents [72, 73], bone-disorder drugs [74, 75] and sensitizers for photodynamic therapy [76, 77]. Intravenously administered fullerene derivatives exhibited no acute toxicity in mice [78]. Since fullerenes are retained in the body for long periods, their chronic toxicities remain to be determined [78]. Therefore, in order to develop fullerene-based drugs or drug carriers, chemical and/or physical modifications that alter fullerene absorption/excretion profiles will be essential. There are several reports to date that mention their usefulness as potential drug carriers.

Zakharian *et al*. first reported a fullerene-based slow-release system for an anticancer drug, paclitaxel [79]. Both fullerene and paclitaxel were chemically derivatized for conjugation designed to insert an ester linkage between them. This type of paclitaxel conjugate is known to show anticancer activity after being released from fullerene by cleavage of the ester linkage, by enzymatic or other physicochemical mechanisms. Incubation of the conjugate in bovine plasma at 37˚C resulted in the slow release of paclitaxel over a period of 4 hours. For aerosol delivery to the lung, the C_{60}-paclitaxel conjugates were further embedded in liposomes through the use of the hydrophobic moiety of C_{60}, while maintaining their anticancer activities.

As described above, fullerenes are chemically active nanoparticles. Isobe *et al*. successfully synthesized a series of cationic fullerenes with several amine groups in various spatial arrangements for optimal gene delivery [80, 81]. As a result of screening based on the transfection efficiency in mammalian cells, a tetraamino fullerene was found to induce gene expression more efficiently than lipofectin, one of the most popular lipid-based transfection reagents.

CARBON NANOHORNS

Single-wall carbon nanohorns (NHs) are recently discovered aggregates of single-wall carbon nanotubes (NTs) with closed ends (Fig. (**3a**)) [82]. The diameter of each tube in NHs is 2-3 nm, which is larger than the 1.4 nm diameter of typical NTs. NHs have a mean diameter of 80-100 nm. When closely looking at the surface of NHs, a large number of horn-shaped tubes can be seen, some of which are kinked (Fig. (**3b**)). These kinked structures and the closed ends of NTs are generated by the presence of pentagonal cells in the graphene sheets consisting of hexagonal cells. The carbon atoms in pentagonal cells are more chemically reactive than those in hexagonal cells. NHs have been reported to be site-specifically modified by reactive oxygen species [83, 84] or sodium amide [85]. In the former case, nanometer-sized pores (<2 nm) with various oxygen functionalities at the edges form in the walls of NHs. Small molecules can then infiltrate into the interior space of NHs through these pores [86]. Furthermore, synthesis of neither NHs nor oxidized NHs (ox-NHs) requires a metal catalyst. Thus, extremely pure materials can be prepared with no potential toxicity derived from metals, which are required for NT preparation [87]. These properties of ox-NHs suggest that they may have a potential advantage as novel carriers in drug delivery systems.

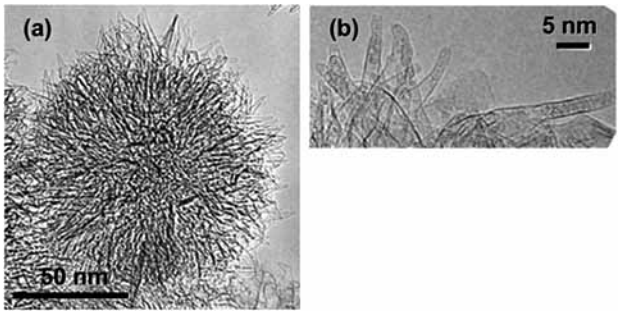


Fig. (3). Transmission electron microscopy images of ox-NH. (a) Image of a whole ox-NH. (b) Magnified image showing the surface area of ox-NHs incorporating fullerenes.

The methods for depositing small molecules in the interior spaces of ox-NHs in the liquid phase have been already established [88]. According to such methods as nanoprecipitation and nanoextraction it is important to use a solvent with which both guest molecules and ox-NHs are equally well solubilized. Our groups have so far revealed that ox-NHs can entrap dexamethasone (DEX) [89], an anti-inflammatory agent, and cisplatin [90], an anticancer agent, in their interior

space, using these methods. Fig. (**4a**) shows Langmuir adsorption isotherms showing the adsorption of DEX by NHs, ox-NHs or ox-NHs heat-treated in H_2 at 1200 ˚C. For all types of NH, the amount of DEX adsorbed gradually increased in a dose-dependent manner. The amount of DEX adsorbed by ox-NHs in a 0.5 mg/ml of DEX solution was determined to be 200 mg for each gram of ox-NHs, which was approximately six times larger than that obtained for unoxidized NHs. H_2 treatment of ox-NHs, which has been shown to reduce the oxygen functional groups at the pore edges, had only a minor effect on the DEX-binding capacity of ox-NHs. These observations strongly suggest that DEX is deposited in the interior space of ox-NHs through the nanometer-sized pores.

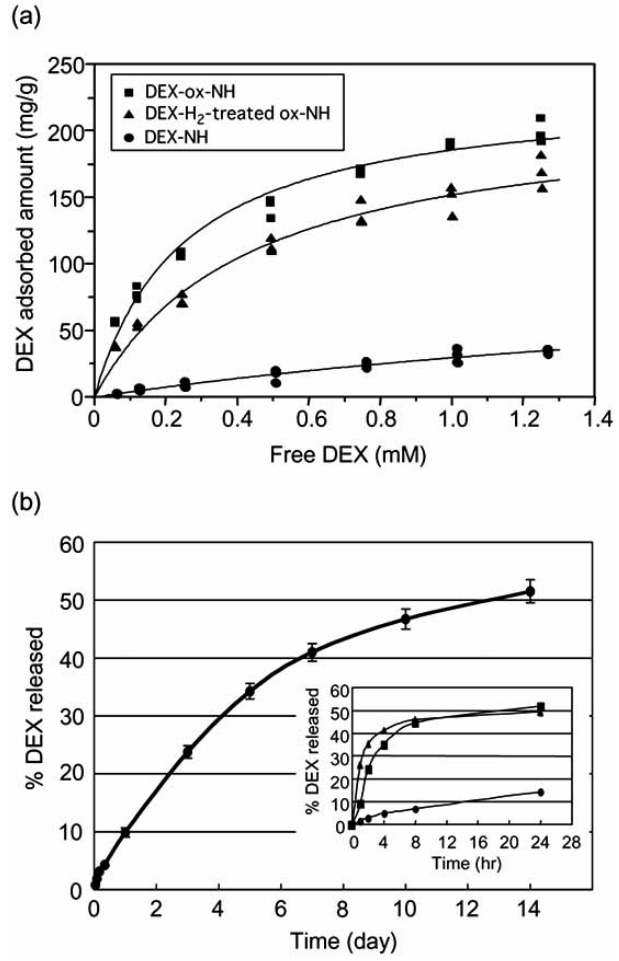


Fig. (4). Adsorption and release of DEX by ox-NHs. (a) Langmuir adsorption isotherms showing the adsorption of DEX by ox-NHs, H_2-treated ox-NHs, and NHs: plotted is the amount of DEX adsorbed *vs*. the steady-state drug concentration. (b) Cumulative DEX release profile of ox-NHs in PBS at 37 ˚C. Total amounts of DEX released up to the indicated times, expressed as percentages of the total DEX bound to DEX-ox-NHs. The inset shows the cumulative release of DEX in PBS (closed circles), RPMI1640 cell culture medium (closed squares), and α-MEM cell culture medium supplemented with 5% FBS (closed triangles) at 37 ˚C.

Controlled-release of drugs from a drug-carrier complex is one of the essential requirements of drug delivery systems. As shown in Fig. (**4b**), DEX-ox-NH complexes were found to slowly release DEX into phosphate-buffered saline (PBS). This slow release continued for at least two weeks. When DEX-ox-NH complexes were immersed in cell culture media instead of PBS, the initial release rates were significantly increased (Fig. (**4b,** inset)). One possible mechanism for these enhancements is that various organic compounds in cell culture medium might be competitively adsorbed to ox-NHs, or they may enhance the solubility of hydrophobic DEX in media.

It was also confirmed that DEX released from these complexes is biologically active. DEX exerts its effects by binding to glucocorticoid receptors in the nucleus, which then activate gene transcription in a DEX-dependent manner [91]. Treatment of mammalian cells transfected with a DEX-dependent reporter plasmid with DEX-ox-NHs for 12 hours activated luciferase expression dose-dependently, whereas ox-NHs induced no activation (Fig. (**5**)). When evaluating the activation level and the DEX-releasing profile during the initial 12 hours, the released DEX was found to retain its biological activity.

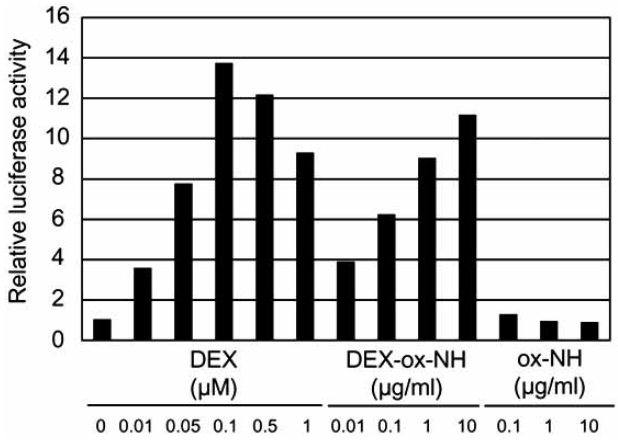
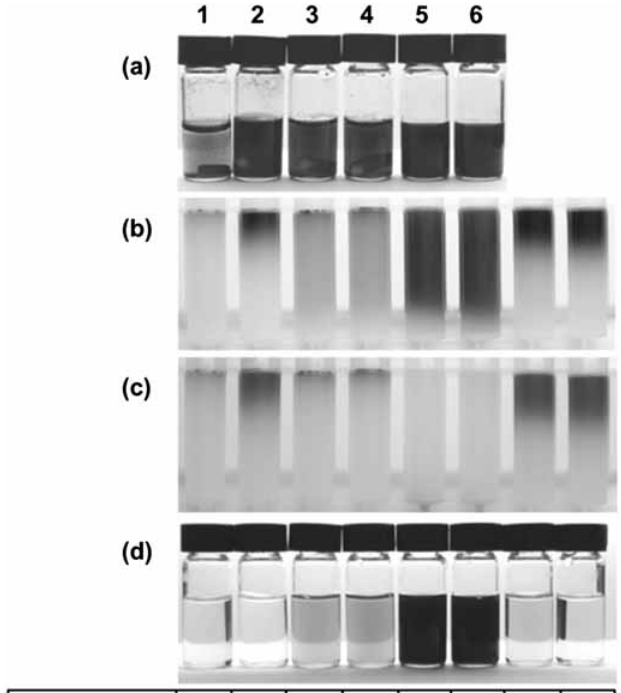


Fig. (5). Effects of DEX-ox-NHs and ox-NHs on glucocorticoid receptor-dependent transcriptional activity. Mammalian cells were transfected with a reporter plasmid and treated with DEX, DEX-ox-NHs, or ox-NHs for 12 hours.

Ox-NHs are highly insoluble in aqueous media and readily self-assemble into agglomerates of micrometer size. Particles with a diameter of more than 4 μm may cause vascular occlusion in the human body [92]. Thus, we developed a procedure for dispersing ox-NHs in aqueous solution using an amide-linked polyethylene glycol-doxorubicin (PEG-DXR) conjugate [93]. With its two aromatic rings, DXR is expected to interact with the surfaces of ox-NHs *via* π-π and hydrophobic interactions. As shown in Fig. (**6a**), treatment of ox-NHs with two kinds of PEG-DXR, in which the average molecular weight of the PEG moieties was either 5000 Da (5PEG) or 20,000 Da (20PEG), yielded well-dispersed ox-NHs (lanes 5 and 6), whereas untreated ox-NHs (lane 1) and ox-NHs treated with either DXR (lane 2) or PEG (lanes 3 and 4) were precipitated. When loaded onto a gel filtration column pre-equilibrated with H_2O, PEG-DXR-treated ox-NHs passed through the column (Fig. (**6b**)) and were completely eluted with H_2O (Fig. (**6c, *d***)), which further supports



ox-NH (250μg)	+	+	+	+	+	+	+	+
DXR (1eq)		+					+	+
5PEG (4eq)			+					
20PEG (4eq)				+				
5PEG-DXR (4eq)					+		+	
20PEG-DXR (4eq)						+		+

Fig. (6). Evaluation of the water solubility of PEG-DXR-treated ox-NHs using gel filtration chromatography. (a) Photographs of vials containing the ox-NH suspension (0.1 mg/ml) after the solubilization procedure under various conditions. (b) Photographs of gel filtration columns after loading with the ox-NH suspensions. (c) Photographs of gel filtration columns after eluting with water. (d) Photographs of vials containing the eluates.

the increased water solubility of PEG-DXR-treated ox-NHs. Elution of ox-NHs was abolished by the co-addition of DXR and PEG-DXR, indicating that PEG-DXR is anchored to ox-NHs by its DXR moiety as expected (Fig. (**7**)).

The average diameter of hydrated PEG-DXR-treated ox-NHs was determined to be 174 nm by dynamic light scattering analysis. It should be noted that this diameter is within the range of 120-200 nm that is escapable from being rapidly trapped in liver and spleen [4, 94]. In addition, due to the "enhanced permeability and retention" effect [9, 10], long-circulating nanoparticles of this size have been recognized to accumulate within solid tumors. We have already confirmed the DXR-dependent antiproliferative activity of PEG-DXR-treated ox-NHs on human cancer cells *in vitro*.

The solubilization procedure described here is accompanied by the deposition of the anticancer drug on the outer surface. As mentioned earlier, the interior space of ox-NHs can serve as a carrier for the anticancer drug cisplatin [90]. Therefore, ox-NHs have the potential to function as double reservoirs for two different drugs; one on the inside and one

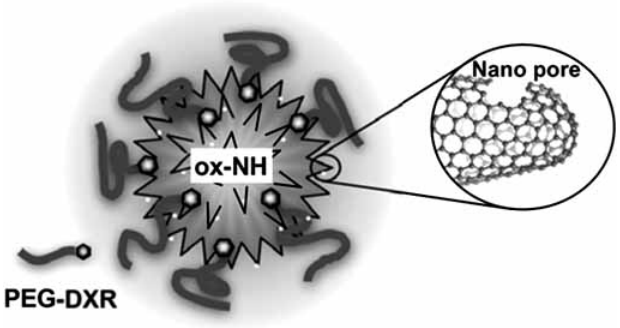


Fig. (7). Possible structure of PEG-DXR-conjugated ox-NH.

on the outside. This characteristic of ox-NHs is very important since multidrug therapy is often used in cancer patients.

CONCLUDING REMARKS

In the present review, we focused on the feasibility of inorganic nanoparticles as drug carriers and discussed recent advances in the development of four intriguing nanoparticles: iron oxide nanoparticles, gold nanoparticles, fullerenes, and carbon nanohorns. All of these inorganic nanoparticles have potential advantages for targeting and controlled-release, which are the two of the most important characteristics of drug carriers. Such advantages are derived from their structural and/or physicochemical properties, and are seldom observed in organic nanoparticles. On the other hand, we must not forget their potential risk for adverse event as described below.

Recently, increasing concerns have been raised about the toxicities of nanoparticles. Among the nanoparticles featured in this review, however, iron oxide nanoparticles have been clinically approved as contrast agents for magnetic resonance imaging [16]. Gold nanoparticles have been generally recognized to have low toxicity [95] except for one report described above [49]. Fullerenes showed neither cellular toxicity toward mammalian cells [96] nor acute toxicity in mice [78], unless derivatized or modified [97]. Ox-NHs accumulated in spleen and kidney within 30 min after intravenous administration to mice, but did not cause any abnormalities at least for the following two weeks [98]. Future studies should clarify their long-term effects and metabolism in the body.

In terms of active targeting, superparamagnetic iron oxide nanoparticles are extremely attractive, as described in this review [27, 32]. Although there are many reports regarding immunoliposomes, in which liposomes and antibodies against a specific cell surface antigen are conjugated to achieve active targeting, their therapeutic potential is not as effective as was expected [99, 100]. Therefore, iron oxide nanoparticles could be the first example of clinically practical drug carriers or elements for active drug targeting.

For controlled-release of drugs, inorganic nanoparticles showed unique properties. Gold nanoparticles can achieve controlled release in response to near-infrared irradiation [57, 61]. In most organic drug carriers, including liposomes and lipid microspheres, the mechanisms of release of drugs are drug diffusion, particle erosion, particle degradation,

polymer swelling, or drug diffusion through a membrane or a wall; in other words, it is passively controlled. In this sense, it is safe to say that gold nanoparticles have the potential to exhibit active controlled release induced by exogenous stimuli. Since metal crystals can be deposited in the interior spaces of ox-NHs [98, 101-103], ox-NHs could be endowed with the properties of gold nanoparticles as well as iron oxide nanoparticles. In addition, a novel type of drug release by ox-NHs could be developed by introducing "nano caps" for their nanometer-sized pores to control drug release through the pores (Fig. (**7**)) [104, 105].

Nanomedicine covers multifaceted fields and interdisciplinary cooperation among pharmaceutical and medical sciences, biopolymer and material sciences, tissue engineering and the science of clinical practices is important. The development of nanomedicine will be at least partly dependent on the rational design of nano-scaled materials based on a better comprehension of pharmacology and biological processes including metabolism. Since raw inorganic nanoparticles have only low biocompatibility as drug carriers, they should be modified by conjugation with organic materials without impairing their intrinsic properties. Mutual interactions between inorganic and organic material sciences on the nano scale, and interdisciplinary technological cooperation of different research areas, would lead to a breakthrough in the development of drug delivery systems by nanomedicine.

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