Biosafe Nanoscale Pharmaceutical Adjuvant Materials

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Abstract

Thanks to developments in the field of nanotechnology over the past decades, more and more biosafe nanoscale materials have become available for use as pharmaceutical adjuvants in medical research. Nanomaterials possess unique properties which could be employed to develop drug carriers with longer circulation time, higher loading capacity, better stability in physiological conditions, controlled drug release, and targeted drug delivery. In this review article, we will review recent progress in the application of representative organic, inorganic and hybrid biosafe nanoscale materials in pharmaceutical research, especially focusing on nanomaterial-based novel drug delivery systems. In addition, we briefly discuss the advantages and notable functions that make these nanomaterials suitable for the design of new medicines; the biosafety of each material discussed in this article is also highlighted to provide a comprehensive understanding of their adjuvant attributes.

Keywords

Drug Delivery; Adjuvant Nanomaterials; Biosafe; Multifunction; Nanoparticle; Controlled Release

INTRODUCTION

Nanoscale materials, as a new class of pharmaceutical adjuvants, have provided many strategies for designing and fabricating novel medicines with a variety of advantages over conventional medicines.[1–4] In the past decades, numerous adjuvant materials were

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developed for drug delivery but few of them went further to clinical use. There are several reasons for this uncomfortable fact and we summarize these challenges in designing biosafe and efficient drug delivery systems as: (1) Inherent toxicity of adjuvant materials; (2) Drug leakage takes place before reaching the lesion; (3) The unfavorable pharmacokinetics, such as rapid elimination and poor stability in blood environment; (4) The non-targeted drug delivery *in vivo*. Therefore, the desired characteristics of pharmaceutical adjuvants are as shown in Figure 1. First, the adjuvant materials should be non-toxic as they are selected with an aim toward human health care. Meanwhile, stimulus-responsive materials that can provide controllable drug release are preferred. Drugs could be protected by the adjuvants through encapsulation, integration, or conjugation before they reach the disease site. After administration, adjuvants and drugs should be stable in physiological environment, avoiding from aggregation and reticuloendothelial system (RES) clearance. Surface coating and modification of drug-loaded nanoparticles, such as PEGylation, can improve their stability, resulting in a longer circulation time.[5,6] Finally, targeted drug delivery, both active targeting by targeting ligands and passive targeting by taking advantage of the enhanced permeability and retention (EPR) effect (an effect that nano-sized particles and macromolecule drugs tend to accumulate in tumor site rather than in normal tissue due to hypervasculature and little recovery of tumor blood/lymphatic vessels), are desired in pharmaceutical research.[7,8] Although nanomaterial's biosafety remains controversial and bioeffect mechanism needs further study, their unique attributes, such as controllable size and shape,[9–11] alternative surface modification,[12–14] high surface area to volume ratio and environmentally-responsive structural deformation,[15–17] still attract great interest from researchers and have enabled them to design new medicines with more functions, including controlled release of drugs in specific physiological environments, targeted delivery of drugs to lesions, longer circulation time, and better loading efficiency of insoluble drugs.[18–21]

Higher accumulation of small anticancer drug molecules in the tumor site can be achieved by encapsulating the drug into nanocarriers, because nanoparticles show preferred retention in tumor as a result of EPR effect.[22,23] Targeted drug delivery can be achieved by modifying the surface shell of drug-loaded nanocarriers with ligands which specifically bind to disease markers. Figure 2 illustrates the EPR effect and targeted delivery of nanoparticles, in this case anticancer drug doxorubicin (DOX)-encapsulated micelles, *in vitro* and *in vivo*.[24] The non-functionalized micelles (M-Dox) showed accumulation in tumor tissue as a result of EPR effect. This nanoparticle was then functionalized with tumor-penetrating peptide CRGDK, the receptor of which are known as neuropilin-1 (NRP-1) protein and are expressed by tumor vessel and by various human carcinoma cells.[25–28] The tumor-penetrating peptide functionalized micelles (TPFM-Dox) are internalized more efficiently and exhibit better accumulation and penetration in tumor. By grafting small drug molecules into nanocarriers (nanoparticles, nanorods, nanocages etc.), it is possible to realize a higher loading capacity, and also to deliver insoluble drugs.[29,30] Moreover, nanomaterials can be designed to release payloads triggered by environmental changes. In cancer treatment, the slightly altered acidic tumor microenvironment can be used to trigger drug release.[31,32] These strategies can help to optimize the biodistribution of anticancer drugs, so that more drug accumulates in the tumor while less is located in the normal organs and the anticancer efficacy is improved with less unwanted side effects. If all these features can be combined in

one nanoparticle system, a delivery system could be generated which acts as a multifunctional drug delivery system.

Traditional medical research has focused on finding and screening new chemical entities (NCEs), with the aim of curing the disease with one new drug. However, this method is expensive, time consuming, and full of unknown risks; in addition, the efficiency is very low. It usually takes more than 10 years, and thousands of candidates were tested. It is possible that only one chemical entity will gain access to the market. Some of the eliminated compounds may have had better efficacy than the final winner, but severe side effect or poor biodistribution prevented them from completing the screening process. Even the winner may have fatal drawbacks, like Paclitaxel (PTX). Due to its poor solubility in water, it was first sold in a formulation employing Cremophor EL and ethanol as solvents, which caused serious side effects in patients.[33] It was later discovered that PTX and albumin could form nanoparticles in solution, and the efficacy was greatly improved without significant side-effects.[34,35] Thus, nanomaterial-based formulation can be used to increase the efficacy of chemotherapeutic agents and reduce the side-effects.

Biosafety is a major issue in the design of novel drugs and researchers must give careful consideration to this when developing nanoscale materials for medical research. The physicochemical properties of nanoparticles, such as size, shape, charge, colloidal stability, and their interaction with environmental compounds, are related to their potential toxicity.[36–39] When nanotechnology was first emerging in the 1980s, the biosafety of nanomaterials, including genotoxic and cytotoxic effects, toxicity to the immune system, *in vivo* biodistribution and clearance, and the long-term effects of treatment, was unclear and more studies were needed to prove that nanomaterials are biocompatible and biosafe.[40,41] Biodegradable and biogenic nanoscale materials, such as proteins, nucleic acids, and phospholipids, are commonly used as pharmaceutical adjuvants. The two Food and Drug Administration (FDA) approved nanomaterial-based drug formulations, Doxil® and Abraxane®, employed organic nanomaterials (lipid in Doxil® and protein in Abraxane®).[42,43] However, some of them may cause immune response (especially extrinsic proteins) and should be tested before clinical use. Inorganic nanomaterials, like silica and gold, are also employed as pharmaceutical adjuvants in research because there is evidence that they are biosafe.[44] However, inorganic materials undergo biodegradation issue and clearance problem, and much research focusing on these two aspects has been done.[45–47] How does inert materials, such as gold and carbon, are degraded and cleared from body is the major concern before their clinical application. Some research work also implied that some inorganic nanomaterials would cause genomic instability, inflammatory response, and protein phosphorylation.[48–50] In conclusion, both organic and inorganic nanomaterials have their advantages and drawbacks when considering biosafety issue. Neither of them should be neglected when talking about biosafe nanomaterials. The concept "biosafe" in this review does not mean "no harm at all," but indicates that the nanomaterials we talked about are with low toxicity and immunogenicity, would not cause severe damage *in vivo* at their applicable dose, and have acquired recognition as potential pharmaceutical adjuvants from most researchers.

In this article, we will review recent progress in biosafe nanomaterial-based pharmaceutical research, using representative organic, inorganic and hybrid materials as examples (Fig. 3). We also highlight the advantages and remarkable functions that make these nanomaterials ideal for use in medicine design.

ORGANIC NANOSCALE MATERIALS EMPLOYED FOR PHARMACEUTICAL DEVELOPMENT

Polymers

Polymers are an important class of nanomaterial in the nanomedicine field due to their ability to encapsulate and protect cargoes and to respond to specific extrinsic stimuli.[51–53] They have been widely used in biomedical research, in applications such as drug delivery,[54,55] gene therapy,[56–58] cell imaging,[59,60] and cancer diagnosis.[61] Polymers can be classified into two categories, natural polymer and synthetic polymer, both of which are important pharmaceutical adjuvant materials. Chemical structures of frequently used polymers in nanomedicine design are listed in Figure 4.

Natural Polymers—Biocompatible natural polymers have attracted great attentions of researchers, since they have many advantages such as low toxicity and biodegradability.[62,63] The most widely studied polymers among them are chitosan and its derivatives. And in order to achieve various functions, many modifications have been applied on the natural polymers.[64]

Chitosan: Chitosan, a natural linear polysaccharide, has been used to synthesize nanoparticles for drug delivery and tissue engineering due to its superior biocompatibility and versatile chemical properties.[65–68] Chitosan nanoparticles will degrade into non-toxic compounds under the effect of lysozyme. These naturally synthesized biomaterials are biocompatible and biodegradable and are relatively easy to obtain at low cost.[69] Chitosan nanoparticles can also be designed to load hydrophobic agents by introducing hydrophobic molecules to chitosan. Therefore, chitosan-based nanoparticles have been employed for delivery of several protein drugs and anticancer chemical drugs, including insulin,[70] DOX,[71] cisplatin,[72] and docetaxel.[73] However, chitosan is only soluble in acidic solvents, resulting in the instability of nanoparticles in neutral or alkaline conditions.[74,75] Fortunately, chitosan provides chemical functional groups which make chemical modification on chitosan possible. A chemical modified chitosan, *N*-imidazolyl-O-carboxymethyl chitosan (IOCMCS), was introduced by Bi and co-workers to improve the solubility of chitosan in water and make the IOCMCS-formed nanoparticle positively charged.[76] Under the N/P ratio of 5, IOCMCS could efficiently bound with plasmid DNA. It also showed higher transfection efficiency and lower cytotoxicity compared to polyethyleneimine (PEI) and Lipofectamine™ 2000. This work is one of the various successful attempts to make chemical modification of chitosan to break the obstacle of solubility and made it a promising material in the field of medicine research. Another interesting work reported by Zhang et al. recently suggested a new application of chitosan.[77] A modified chitosan, *N*-octyl-*O*-sulfate chitosan (NOSC), was applied to assemble into micelle for PTX loading. According to previous report, NOSC was possible to inhibit the key drug resistance-related protein P-glycoprotein

(P-gp, also known as MDR-1).[78–80] Thus, PTX-encapsulated NOSC micelle (PTX-M) could deliver PTX into cells bypassing P-gp. After internalization, NOSC micelle disassembled and inhibited P-gp function, improving PTX retention in cells (Fig. 5(A)). Anticancer efficacy towards HepG2 cell and drug resistance cell HepG2-P of different PTX formulations, including taxol (PTX dissolved in Cremophor EL and ethanol), taxol + NOSC (physical mixture of taxol and NOSC), and PTX-M, was assessed *in vitro* and *in vivo*. Cytotoxicity evaluation result indicated that PTX-M possessed the best cell killing efficacy in HepG2-P cells with the lowest half maximal inhibitory concentration (IC 50) (0.55 μg/mL). Meanwhile, IC 50 of PTX was 16.39 μg/mL (Fig. 5(C)). PTX-M also exhibited the best tumor growth inhibition efficacy *in vivo* as tumor growth was significantly controlled after PTX-M injection (Figs. 5(D), (E)). Further study of PTX accumulation in cells and tumors of different formulations revealed that PTX-M could increase the amount of PTX in cells and tumor tissues which may be a result of the dysfunction of P-gp (Figs. 5(B), (F)). Therefore, drug resistance of HepG2-P cell was successfully overcome *in vitro* and *in vivo* by encapsulating PTX into P-gp inhibiting chitosan derivative NOSC. NOSC in this work is a P-gp inhibitor as well as a drug carrier. More interesting work could be developed basing on this material.

Synthetic Polymers—Compared to natural ones, synthetic polymers are more designable, and can also be biocompatible by using appropriate compounds while synthesizing.[81,82] The method of synthesizing can be various, such as polymerization, covalently binding, and physical assembly. Optimizing polymers to be more functional and biocompatible is now the most important research field of polymer science and attracts many scientists' attentions.[83–85]

<u>PEG and Its Derivatives:</u> Polyethylene glycol (PEG) is another important pharmaceutical adjuvant in the nanomedicine research field. Most unmodified nanostructures, such as liposomes, micelles, and nanocrystals, usually have a high rate of uptake by the RES, leading to significant accumulation of nanomaterials in the liver and spleen.[86] PEGylation, the process of covalently attachment of PEG to another molecule, can increase the hydrodynamic size of nanoparticles and hinder the interaction between nanoparticles and plasma proteins, resulting in reduced immunogenicity. Nanoparticles will then show improved biocompatibility, with ultra-long circulation times and high water solubility after PEGylation.[87] Nowadays, many PEGylated medicines, especially PEG-liposomes and PEG-proteins, have got their FDA approval because of prolonged circulation time and reduced immunogenicity.[88] Figure 6 summarized several PEG-protected nanoparticles with improved pharmacokinetics. PEG was conjugated with another FDA-approved adjuvant materials, poly(lactic-co-glycolic acid) (PLGA), and reported by Pei and co-workers.[89] I[125]-labeled bovine serum albumin (BSA) was loaded in PEG-PLGA and PLGA nanoparticles to trace circulation and biodistribution of two nanoparticles *in vivo*. The PEG-PLGA nanoparticle exhibited longer blood circulation time than PLGA nanoparticle. Moreover, PEG-PLGA nanoparticles remained a much higher concentration in blood than PLGA nanoparticle 12 h after intravenous injection (Figs. 6(A)–(C)). PEG is also important surface coating agent of inorganic nanomaterials. PEG-protected gold nanorods (Au NRs) obtained by replacing CTAB with methyl-PEG thiol were stable in serum, while CTAB-protected Au

NRs aggregated.[90] Blood clearance test showed that PEG-protected Au NRs has a very high half-life of ~ 17 h. 72 h after intravenous injection, PEG-Au NRs were still visible in tumor site by thermal imaging (Figs. 6(D)–(F)). In another work, Dai et al. functionalized single-walled carbon nanotubes (SWNTs) with a branched PEG polymer, named as $PMHC_{18}$-mPEG.[91] The blood circulation time of PEG polymer-protected SWNTs in mice upon intravenous injection was greatly prolonged ($t_{1/2}$ = 22.1 h) compared to the previous record ($t_{1/2}$ = 5.4 h). The $PMHC_{18}$-mPEG was also compared with DSPE-PEG in SWNT protection *in vivo* and $PMHC_{18}$-mPEG-coated SWNT showed longer half-life in blood (Figs. 6(G)–(I)). PEG holds great potential in imaging probe protection *in vivo*. Mulder et al. investigated the biocompatibility and pharmacokinetics of quantum dots (QDs, an important imaging probe) containing silica nanoparticles after PEG coating.[92] Both bare and PEG-coated nanoparticles were injected into mice and blood sample were obtained several minutes after injection. Then fluorescence intensity, resulting from QDs, was determined and the results showed that bare nanoparticles were rapidly cleared from blood. Meanwhile, PEG-coated nanoparticles remained in blood and exhibited fluorescence signal even 240 min post injection. Biodistribution analysis demonstrated that less PEG-coated nanoparticles were uptake by RES organ than bare nanoparticles (Figs. 6(J)–(L)). Reduced adsorption of protein on PEG corona is the main mechanism of PEG protection. Griesser et al. provide some evidence to support this viewpoint.[93] Nonspecific adsorption of proteins on naked and PEG-protected silica nanoparticles was determined by X-ray photoelectron spectroscopy (XPS) and Fourier transform infrared spectroscopy (FTIR) analysis, and the results confirmed that protein was hardly adsorbed by PEG-protected nanoparticles (Figs. 6(M)–(O)).

PLA and Its Derivatives: Poly(lactic acid) (PLA) and PLGA are the most widely used polymers as they have been approved by the FDA.[94,95] PLA- and PLGA-based nanocarriers were developed to deliver various drugs;[96,97] however, the rapid burst of cargo release without any specificity limited further *in vivo* application due to the low drug concentration at the desired site.[98,99] Thus, subsequent work focused on improving the specific release of drugs at the disease site by linking drug release with a specific biological process. Scientists have now successfully synthesized polymeric nanoparticles which are responsive to specific external stimuli, such as light,[100] temperature,[101] and ultrasound,[102] especially to inherently occurred low pH in tumor microenvironment and endosome.[103] Compared to the neutral pH in healthy tissues, the lower pH in the tumor microenvironment and in endosomes can be exploited so that nanoparticles release their drug payloads in the right place.[104] The most widely used strategy to design pH-responsive polymeric nanoparticles is to introduce a protonation group, such as carboxylic acids and tertiary amines, into the polymer structure which can be ionized at different pH values. Another strategy to achieve pH-responsive polymeric structures is to use acid-labile functional groups which can be hydrolyzed to reveal a new hydrophilic group; the polymer then becomes water-soluble and the nanoparticle structure decomposes, thus releasing the loaded agent. For example, Fréchet et al. synthesized acetal-derivatized dextran-based particles which degraded in a pH-dependent manner (Fig. 7(A)).[105] At pH 7.4, the nanoparticles possessed a half-life of 360 h, as judged by measuring free dextran, but when the pH was adjusted to 5, the nanoparticles became unstable and the half-life decreased to 10 h. The ultra-long half-life at neutral pH and significantly decreased stability at acidic pH are ideal for pH-sensitive drug delivery.

Nanoparticles would be extremely stable in normal tissue and response sensitively once they get to the disease site. Another merit of this approach is that both hydrophobic and hydrophilic agents were successfully encapsulated within the nanoparticles and, as dextran is biocompatible, these particles are non-toxic to cells *in vitro*.

PVP and Its Derivatives: Poly(*N*-vinylpyrrolidone) (PVP) has a lot of properties which make it an outstanding candidate as pharmaceutical adjuvant material, such as solubility in water, very low toxicity, good biocompatibility, and high complexation ability.[106] PVP was initially used as a blood plasma substitute and then be widely used in medicine and pharmacy.[107] The highly hydrated structure of PVP suppressed its interaction with immune system and made PVP a good choice as coating agent to reduce immunogenicity of nanomaterials.[108] The slow degradation of PVP under ultraviolet (UV) or ultrasound irradiation is another promising aspect.[109] It has also been reported that PVP can cause intracellular vesicular swelling and diminish the fusion of endosomes to lysosomes.[110] These unique features of PVP make it a good drug and gene carrier. However, the non-biodegradability of macromolecular PVP will lead to accumulation *in vivo*. To deal with this problem, PVP with molecular weight below the kidney threshold was synthetized through various methods. Saxena et al. reported a cross-linked PVP nanoparticle of size less than 100 nm which can encapsulate plasmid DNA.[111] The result showed these particles have high transfection efficiency both *in vitro* and *in vivo*. This synthesis method provided a low molecular weight PVP nanoparticle which was a promising vehicle for drug and gene delivery.

Amphiphilic Copolymers: Copolymers, especially amphiphilic copolymers, are the most common polymers in nanotechnology.[112–114] Amphiphilic copolymers can self-assemble into micelles or nanoparticles in aqueous solution, with a hydrophobic core inside and a hydrophilic shell outside. Each chain of the copolymer can also provide different functions, to make the particle multifunctional. For example, Guo et al. synthesized a copolymer composed of three polymers, methoxy polyethylene glycol-block-(polycaprolactone-graft-poly(2-(dimethylamino)ethyl methacrylate)) (PEG-*b*-(PCL-*g*-PDMAEMA)).[115] The polycaprolactone (PCL) backbone can (1) form a hydrophobic core to carry hydrophobic drugs such as PTX and DOX; (2) improve drug/gene delivery efficiency by enhancing the cellular uptake through effective interaction between the hydrophobic chains and cell membrane. The side chain poly(2-(dimethyl-amino)ethyl methacrylate (PDMAEMA) is positively charged which can condense plasmid DNA and provide endosome escaping capability. As described above, PEG, the biocompatible part of backbone, forms a hydrophilic shell to protect the particle and decrease the surface positive charge. This well designed amphiphilic copolymer can self-assemble into nanoparticle and shows high gene delivery efficiency *in vitro*.

Lipids

Lipid is a group of natural molecules including fats, sterols, fat-soluble vitamins, glycerides, phospholipids, and others. Among these lipids, phospholipids are widely employed as nanoscale pharmaceutical adjuvants in the form of liposomes and micelles and will be highlighted in this review.[116,117] Phospholipids are amphiphilic compounds, with a

hydrophilic polar group and a hydrophobic chain, which mimic the components of naturally occurring membrane bilayers.

The best-known lipid-based nanostructures are liposomes, in which an internal aqueous core is separated from the bulk aqueous phase by a closed lipid bilayer shell.[118] Micelles, a nanostructure similar to liposomes, are usually composed of closed lipid monolayers with a fatty acid core and a polar surface, or a polar core with fatty acids on the surface (inverted micelle). Nowadays, many amphiphilic polymer-based micelles have been developed as a result of their more versatile structure and function compared to that of lipid.[119,120] Liposomes are capable of carrying both hydrophobic and hydrophilic drugs at the same time. The hydrophobic drugs can be integrated into the fatty acid shell while the hydrophilic drugs can be loaded into the aqueous core. In contrast, either hydrophobic or hydrophilic drugs can be encapsulated into micelles, determined by the core's polarity. Micelles are much smaller in size (1–20 nm) than liposomes (100–200 nm); however, the size of micelles is more uniform. The surface of liposomes can also be functionalized with a variety of ligands and reporters, such as antibodies,[121] nucleic acids,[122] and luminescent sensors.[123] The methods for liposome preparation are well developed and the process is very easy to control. Under specific conditions, amphiphilic lipids will self-assemble into liposomes. By adjusting the formulation (lipid ratio, source) and changing the synthesis conditions (temperature, solvent, ion concentration, and sonication), the size, uniformity, and stability of liposomes can be manipulated. The first FDA-approved nanodrug, known as Doxil® in the market, is a liposome loaded with the anticancer drug DOX. DOX is commonly used in the treatment of a wide range of cancers; however, the adverse side effect is life-threatening heart damage.[124] Using the Doxil® formulation, the drug circulation time is prolonged and more drug accumulates in the tumor site as a result of the passive targeting of liposomes to the leaky tumor vasculature.[125]

Lipids can also be grafted onto other nanomaterials as surface coatings for further modification, better stability, and improved biocompatibility.[126,127] PLGA nanoparticles are great siRNA carriers due to their stability and low toxicity.[128] However, the transfection efficiency of PLGA nanoparticles is low and not comparable to that of cationic lipid nanoparticles.[129] Thus, DeSimone et al. combined them and developed lipid-coated PLGA nanoparticles that could deliver multiple siRNAs for the treatment of prostate cancer (Fig. 7(B)).[130] The encapsulation efficiency was high (32–46%) and target gene expression was greatly down-regulated.

Lipids can be classified according to their surface charge: neutral lipids, anionic lipids, and cationic lipids. Cholesterol is separately introduced due to its special role in lipid-based nanomaterials. Table I shows the commonly used lipids in pharmacy research.

Neutral Lipids and Anionic Lipids—Neutral lipids and anionic lipids are similar in character and application. They are the main composition of conventional liposomes for pharmaceutical applications. Phosphatidylcholine (PC), phosphoethanolamine (PE), 1,2-dioctanoyl-sn-glycerol (DG), *L-α*-phosphatidic acid (PA), *L-α*-phosphatidylglycerol (PG) and their derivatives are commonly used in various liposomes. For instance, PC is a major constituent of cell membranes, which have been shown to stabilize membranes and can form

a lamellar structure that is insensitive to the pH.[131] Due to their membrane structure and neutral or negatively charged surface, neutral and anionic liposomes can decrease the non-specific protein adsorption and remain longer in blood circulation *in vivo*. Awasthi et al. prepared PEGylated neutral and anionic liposome-encapsulated hemoglobin (LEH).[132] The neutral and anionic LEH showed long-lasting ability and the circulation half-life of LEH was improved after PEGylation. Besides, neutral and anionic lipids can be modified and cooperated with other functional groups. We have recently reported a novel pH-sensitive liposome which can circumvent the DOX-resistance of cancer cells.[133] One way that cells develop multi-drug resistance (MDR) is by expressing high levels of certain membrane proteins, such as P-gp, which mediates efflux of intracellular drugs.[79,80] We developed a system to create a sudden increase in the intracellular drug level, thus killing the cell before drug export can occur. This liposome is composed of neutral lipids hydrogenated soy phosphatidylcholine (HSPC) and PEGylated 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE). We loaded liposomes with ammonium bicarbonate and DOX and demonstrated that at pH 5.0, the bicarbonate ions decomposed and released CO_2 gas, which raised the internal pressure and ruptured the liposomes. When these liposomes reached the endosomes (pH ~ 5.0) in MDR breast cancer cells, they released all their DOX at the same time and the intracellular drug concentration reached the effective concentration immediately. Thus, resistant cells were killed before their MDR mechanisms had time to work. This work illustrates a new strategy for designing and synthesizing pH-sensitive liposomes.

Cationic Lipids—Cationic lipids, for example ethyl phosphocholine (ethyl PC), 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA), and cationic cholesterol, have cationic hydrophilic head to attract negatively charged molecules, especially nucleic acids, and form complexes. In this way, cationic lipids can be used as nucleic acid carriers and are regarded to be the most promising non-viral gene vectors.[134] Tenchov et al. synthesized various ethyl PCs with different hydrophobic moieties, and investigated their transfection efficiencies in human umbilical artery endothelial cells (HUAEC). The result showed that the compounds exhibited very high transfection efficiencies.[135] Through the investigation of liposome phase-conversion during transfection process, they found that high transfection efficiency was resulted from enhanced liposome fusion with cellular membranes. This work could be useful to assess the effect of cationic lipid carriers and for further design of new and effective gene delivery system.

Cholesterol—Cholesterol is an essential structural constituent of animal cell membranes. The addition of cholesterol to lipid bilayers decreases its permeability to water and thus drug leakage is prevented.[136] Cholesterol is also incorporated into the liposome layers to increase their ordered state as it has an additional stabilizing effect.[137] It limits the mobility of acyl chains of phospholipids, rigidifies the membrane in the liquid-crystalline phase, and increases the mobility of these chains while in the gel phase. This character decreases the phase transition temperature of lipid membranes, a parameter that is important for the relative fluidity and mobility of lipid molecules in the bilayer. Thus, cholesterol is a regulator in lipid bilayer and plays an important role in lipid-based nanoparticles.

Proteins, Peptides and Nucleic Acids

Proteins, peptides, and nucleic acids have many different roles in the development of nanomedicines. They can act as drugs (e.g., interferon, insulin, tesamorelin, siRNA), target ligands (e.g., aptamer, antibody, nuclear localization signal peptide), and carriers (e.g., albumin, DNA origami). As they are the most common materials in the human body, their biodegradability is in no doubt. Much higher systemic concentrations of proteins, peptides, and nucleic acids can be administered than that of inorganic nanoscale materials. As pharmaceutical adjuvants, proteins, peptides, and nucleic acids are irreplaceable because of their targeting ability. Illustrative examples of targeting ligands, including proteins, peptides, and nucleic acids, are listed in Table II. Metabolism mechanism of them is also clearly studied, making them more advantageous than other materials. However, the disadvantage is that their structures are vulnerable and it is difficult to make modifications. Once the fine-designed structure is changed, they will lose functions and integrity. Another issue that should be addressed is the immunogenicity as immune system will be activated by exogenous proteins and DNA sequences.[138]

Proteins—Self-assembled protein-based nanocages can be considered as natural drug carriers. In nature, some protein structures, such as microtubules, are assembled from multiple copies of protein subunits.[139] Recently, albumin, gelatin, and silk protein-based protein nanoparticles have been developed as drug delivery systems following the successful model of Abraxane®.[140–142] These particles are biosafe and non-antigenic; furthermore, they can realize active targeting as well as passive targeting. For example, albumin can penetrate the microvessel endothelial cells in angiogenic tumor vasculature with the assistance of the albumin-binding protein gp60 and combine with the target protein SPARC which is over-expressed in many tumors.[143,144] Cao and co-workers used protein nanoparticles for active tumor targeting.[145] They employed human ferritin H-chain protein (FTH1) as the framework and combined it with epidermal growth factor (EGF), the ligand of EGFR, a receptor that is over-expressed in many malignant tissues.[146,147] The EGF-FTH1 nanoparticles were specifically internalized by two breast cancer cell lines, MCF-7 and MDA-MB-231, but the uptake by normal epithelial MCF-10A cells was negligible. *In vivo* experiments also demonstrated the accumulation of EGF-FTH1 nanoparticles in breast tumors in a mouse xenograft model. Jiang et al. cross-linked the milk protein casein with transglutaminase to form nanoparticles, which were then loaded with cisplatin, a model anti-cancer drug (Fig. 7(C)).[148] In the murine xenograft model, cisplatin-loaded nanoparticles inhibited tumor growth 1.5-fold more effectively than free cisplatin and analysis of tumor sections demonstrated that the casein nanoparticles could penetrate through the tumor tissue and deliver cis-platin to cells far from the vasculature.

Collagen is another important biosafe nanomaterial that should be addressed. It is the main structural protein in animals and accounts for one third of total protein in human.[149] 28 types of collagen have been identified in vertebrate and 90% of the collagen in the body is type I.[150,151] As collagen is the major component of the extracellular matrix (ECM), the biocompatibility of collagen is good.[152–154] In a recent work reported by Cai and coworkers, collagen was linked to silica nanoparticles via disulfide bond as a protein cap to protect encapsulated fluorescence probe FITC.[155] Silica nanoparticle is an excellent reservoir for

drugs, and end-capping of them could protect encapsulated cargos, separate cargos from external environment. As a capping material, collagen has advantages in biosafety. In addition, collagen is full of sulfydryl, which are necessary to form disulfide bond that can be broke by various reducing agents, including cell-expressed glutathione.[156] Then, specific cargo release from nanoparticles in cytoplasm can be realized. This work subtly utilized this property and conjugated collagen with nanoparticle through disulfide bond. Redox-responsive release of cargo FITC from nanoparticles was successfully identified after adding another reducing agent, dithiothreitol (DTT), to the nanoparticle solution. Targeting moiety, lactobionic acid (LA), was also introduced to the collagen-capped nanoparticle and was demonstrated to be effective in cell-specific targeting *in vitro*. Therefore, redox-responsive release and targeted delivery were achieved in this work.

Gelatin, a derivative of collagen, has similar potential as pharmaceutical adjuvant just like collagen. It is widely used in food and pharmaceuticals as gelling agent.[157] Gelation would be easily triggered by increasing the temperature and suddenly cooling of gelatin solution. Nanoparticles protected by a gelatin cap could then be obtained by this method. He, Wang, and co-workers reported a work employing gelatin as a cap to protect DOX-loaded nanoparticles recently.[158] The gelatin cap was successfully grafted to the nanoparticles by temperature-induced gelation and stabled by glutaraldehyde mediated cross-linking. Interestingly, gelatin is a natural pH-sensitive agent and the authors take this advantage and successfully made the gelatin-capped nanoparticles pH-sensitive. As a mixture of protein and peptide, gelatin contains many protonation groups like carboxyl and amine, making it negatively or positively charged determined by the pH value. Moreover, the isoelectric point of gelatin can be modified by removing or retaining the protonation group.[159] Thus, the charge of gelatin can be precisely controlled. In this work, the loaded-DOX was well-protected upon neutral pH and released from the nanoparticles once the solution was adjusted to be acidic. This pH-sensitive behavior was also confirmed *in vitro* by co-localization analysis of DOX and endosome assisted by confocal laser scanning microscopy (CLSM). DOX was first trapped in endosome, exhibiting high co-localization ratio with endosome at 5 h. Then DOX escaped from endosome and entered into cell nucleus at 12 h, indicating that gelatin cap was eliminated from the nanoparticle surface.

Peptides—Peptide, natural outcome from protein metabolism, played a crucial role in biological activity. Accompanied by the progress of peptide synthesis, peptides are more widely used as therapeutics, targeting ligands and drug vectors.[160,161] Especially, peptide presents an advanced and rapid developing in the field of drug delivery. Compared to other drug carrier materials, peptides possess several advantages for drug delivery, including smallness, versatile structures, facile synthesis, biocompatibility, and biodegradability.[162]

Many studies have confirmed the potential and superiority of peptide applied as drug delivery system. For example, Divita and co-workers successfully developed a peptide-based novel method to delivery siRNA into mammalian cells.[163] They designed an amphiphilic peptide (CADY) of 20 residues combining aromatic tryptophan (Trp, W) and cationic arginine (Arg, R) residues. The recombinant peptide forms stable complexes with siRNA, thereby increasing the stability of the complexes forming from peptide and siRNA and improving their transfection efficiency into a wide variety of mammalian cells, notably

including primary cell lines. The siRNA delivery based on the CADY peptide significantly knockdown the target gene at subnanomolar concentration of siRNA. And more importantly, the cytotoxicity results showed that the CADY peptide was non-toxic and entered cells in an endosome-independent way, delivering therapeutics into cells without enzymolysis under lysosome enzymes. CADY peptide can then be used for the delivery of unstable drugs and those whose therapeutic efficacy are compromised due to the lysosome sequestration. Meanwhile, peptides possess a great potential in the chemotherapy drug delivery. Recently, a self-assembled peptide hydrogel formed by peptide and anti-cancer drug taxol was successfully designed by Yang and co-workers.[161] The peptide hydrogels of taxol could markedly inhibit tumor growth and metastasis through local administration of taxol hydrogels. In addition, the peptide-based taxol hydrogels enhanced the dosage tolerance of mice to taxol by reducing the plasma concentration of taxol which is due to the local administration, and finally decreased the side effects of taxol. Peptide hydrogel can be applied for controlled and sustained release of drugs to skin tissues, which is important for wound healing, anti-inflammation, and dermatosis.

Except for drug carrier, peptide also plays an important role as functional agent in drug delivery system. Cell penetrating peptides (CPPs) and pH-sensitive peptides are two major categories of functional peptides. CPPs are a series of peptides which can facilitate cellular uptake, endosomal escape, and nuclear targeting.[164,165] Tat peptide, a CPP derived from transactivator of transcription (tat) of type I human immunodeficiency virus (HIV-1), has been widely used in drug delivery and gene transfection.[166,167] Polyhistidine, as a famous example of pH-sensitive peptide, has a pKa of ~ 6.5. When the pH is turned to be below 6.5, polyhistidine would shift from hydrophobic to hydrophilic.[168,169] Caruso et al. recently successfully combined these two functional peptides in one, named H4R4, and reported its application in nanoparticle-based drug delivery.[170] R4 stands for 4 arginines, and it is mimicking the functional region of tat peptide which is rich of arginine (GRKKRRQRRRPQ). H4 is 4 histidines, and is the pH-sensitive part of the combined peptide. R4, like many other CPPs, is highly hydrophilic and cannot be used alone without conjugation in nanoparticle functionalization. However, conjugation makes it hard to vary the amount of CPP used.[171] Here H4 provides a hydrophobic part and makes H4R4 an amphiphilic peptide under neutral pH. It is simply mixed with a PEG-DOX conjugate and a poly(2-diisopropylaminoethyl methacrylate) (PDPA) homopolymer. PEG-DOX and PDPA can assemble into a nanoparticle with a hydrophilic corona and hydrophobic core, which provide space for the amphiphilic H4R4. In the acidic endosomal compartment, H4 become hydrophilic and H4R4 is released from the nanoparticle. Then, R4 could facilitate the release of PEG-DOX from endosome and its further entrance into cell nucleus. The PEG-DOX loaded in H4R4 mixed nanoparticle exhibits better endosome escape ability and higher accumulation in nucleus than that loaded in non-H4R4 mixed one, confirmed by co-localization analysis. More importantly, the cytotoxicity of the nanoparticle is tunable by adjusting the amount of H4R4 mixed. This work provides us a new sight in the application of peptides. Peptide is versatile in functions and combination of two functional peptides is easy. We may have more multifunctional drug delivery systems in peptide-involved nanoparticles.

Nucleic Acids—Nucleic acids are considered to be fragile due to their instability in physiological environments which contain DNase and RNase. It may seem impossible that nucleic acids could be used as pharmaceutical adjuvants to carry drugs, but the opposite has proved to be true. Compared to the common linear structure, engineered DNA structures can be very stable, and have been used to build nanoparticles for the past 30 years. DNA nanostructures have great potential in the areas of molecular and cellular biophysics, energy transfer and photonics, and diagnostics.[172] Recently, Ding and co-workers successfully applied "origami" DNA nanostructures, made of folded nucleic acid strands, to the drug delivery field (Fig. 7(D)).[173] The electrostatic attraction between DOX and DNA structure was subtly used in this work. The stability issue of DNA was also settled by converting linear strands into origami nanostructure. These self-assembled, spatially addressable, DNA origami nanostructures were very stable in cell lysates, and the DOX loading efficiency was high because the drug was non-covalently bound to the DNA through intercalation. The cellular uptake of DOX was increased by this DNA origami nanostructure, resulting in a better cell killing efficiency. Meanwhile, RNA nanotechnology has achieved rapid development in biomedicine applications in recent years. A packing RNA (pRNA) of bacteriophage phi29 DNA-packaging motor is developed by Guo and coworkers to work as a highly efficient nanovector to carry siRNA (MT-IIA) for cancer therapy.[174] The pRNA/siRNA can protect the siRNA from RNase and keep stable in serum. The messenger RNA (mRNA) levels of MT-IIA and its downstream gene survivin were down-regulated by this pRNA/siRNA complex, resulting in suppressed cell proliferation. Furthermore, the dimmers that were obtained by tagging the pRNA/siRNA complex with folate can be delivered cell–specifically to ovarian cancer cells which express folate receptor. From both examples we can find that stability is the major problem for nucleic acids as pharmaceutical adjuvants. If this can be settled properly, DNA and RNA have a promising future as a potent tool for drug delivery.

Other Organic Materials

Besides polymer, lipid, protein, peptide, and nucleic acid, saccharide is another important nanoscale pharmaceutical adjuvant materials. Cyclodextrins (CDs), a family of compounds made of saccharide molecules forming a ring structure, will be introduced as an outstanding saccharide. They are produced from starch by enzymatic conversion.[175] CDs are composed of 5 or more α-D-glucopyranoside units and typical CDs are formed by 6–8 monomers in a cone shape, known as α-CD (6 monomers), β-CD (7 monomers), and γ-CD (8 monomers) (Fig. 4).[176] Compared to chitosan and other polymers, CDs are commonly used as one of the most versatile aids in pharmaceutical technology to enhance the aqueous solubility and chemical stability of biologically active compounds.[177] Especially by using guest-host chemistry, CDs can introduce the guest functional groups to the host compounds. Ma et al. conjugated β-CDs to a branched PEI as a host unit.[178] Then a guest hydrophobic polymer poly(β-benzyl L-aspartate) (PBLA) was introduced to the host scaffold to mediate the assembly process. The hydrophobic core formed by PBLA serves as a nanocarrier to load hydrophobic drugs, while the positively charged hydrophilic PEI shell is capable of condensing plasmid DNA and achieve its transfection *in vitro*. In this way CDs produce a new way of assembly, and can be used as a new generation of multifunctional nanocarriers.

INORGANIC NANOSCALE MATERIALS AS POTENTIAL ADJUVANTS IN PHARMACEUTICS

Silica

In the past decade, silica-based nanoparticles have attracted more and more attention as pharmaceutical adjuvants.[179] The biocompatibility of silica nanoparticles has also been thoroughly studied in different cell lines, and there is strong evidence that they are only toxic at a high dose.[180] Some recent results indicated that silica nanoparticles are biocompatible in mice after serological, hematological, and histopathological examinations of blood samples and tissues.[181]

Silica Nanoparticles—The most well-developed of these silica-based nanoparticles are mesoporous silica nanoparticles (MSNs) because of their tailored mesoporous structure and high surface area.[182–184] Mesopores can be modified with different chemical groups to be hydrophobic or hydrophilic, enabling MSNs to encapsulate a variety of drugs. Meanwhile, high surface area allows a much higher drug loading capacity compared to that of solid nanoparticles.

By preferentially manipulating the biodistribution of encapsulated pharmaceutical agents in the target organ/tissue, MSN-based systems have the potential to deliver an effective concentration of drug to the lesion at a relatively low systemic drug dose. The adverse side-effects can therefore be minimized without compromising treatment efficacy. To accomplish this goal, the surface of MSNs can be modified with a variety of functional agents, such as polymers, proteins, and stimulus-responsive groups. These surface coatings work as gatekeepers to protect the pharmaceuticals inside the pores and achieve a controlled release.[185,186] β-CD, a saccharide which is responsive to the acidic conditions within endosomes, was employed by Zink and co-workers to work as a nanovalve in MSNs for pH-sensitive drug release.[187] This nanovalve is tightly closed at pH 7.4 and acidic environment is the key to open the valve and release cargos. The leak and rapid burst of drug from nanoparticles are crucial problems for drug delivery and nanovalve is a good strategy to overcome these challenges. Mesopores can be sealed under physiological condition and release drug slowly under certain stimuli. Figure 8 shows three typical nanovalve-based controlled release mechanisms. Gatekeeper molecules are covalently attached to MSNs while plug molecules are then plugged into the gatekeepers through host–guest interaction. In some work only gatekeeper molecules are employed. Initially, cargo molecules are loaded in the channels of MSNs. Drug release can be initiated in three ways:

1. cutting off the covalent bonds between gatekeeper molecules and MSNs;

2. pulling out the plug molecules by interfering host-guest interaction;

3. destroying gatekeeper molecules.

These mechanisms can be designed to be responsive to various stimuli, including pH, light, thermal, ultrasound, oxygen stress, and several enzymes. It is a hot pursued area to develop smart drug delivery systems with nanovalves. Reversible and precisely controlled nanovalves will be the future trend.

MSNs are also good candidates for multifunctional nanoparticles. Nanoparticle-based drug delivery systems are confronting many challenges, including uptake by the RES, interaction with serum proteins, and poor accumulation at the disease site. Thus, researchers have concentrated much effort on integrating several functions into one particle and, as a result, many MSN-based multifunctional nanoparticles have recently been developed.[188–190] Lin et al. functionalized MSNs with folate groups, the receptors of which are highly expressed in human cancer cells (Fig. 7(E)).[191] They demonstrated that the endocytosis of the modified nanoparticles is a receptor-mediated process and can be inhibited by free folic acid. Hyeon and co-workers reported functionalized MSNs that could be used for magnetic resonance imaging (MRI), fluorescence imaging, and drug delivery, thus combining therapeutic and diagnostic functions.[192] DOX was loaded into the pores and the particles were stabilized by coating with PEG to increase the circulation time. The anticancer effect of DOX-loaded nanoparticles was also observed *in vitro* and *in vivo*. In this work, both MRI and PEG coating are employed for integrated multifunction. MRI is superior than fluorescence imaging in clinical use because the latter will photobleach and the imaging depth is only a few millimeters. PEG is the best stealth delivery cover so far as introduced in the polymers section. MSN is a well-established integration platform on which multiple functions could be combined thanks to the versatility and compatibility of MSNs.

Compared to other adjuvant materials, MSNs are superior in various aspects: higher available surface area allows for more functionalization; tailored mesoporous structure provides sealed compartment for hydrophilic and hydrophobic cargos; strong adsorptive capacity results in higher drug loading; toxicity study and previous application as food additive stands for their biosafety. However, we should also pay attention to the hydrolysis of MSNs' backbone (Si–O–Si) caused by acidic pH environment. In addition, unmodified silanol groups on MSNs will interact with red blood cell membrane and cause hemolysis.[193] Further modification of silanol groups and hemolysis test are needed before MSNs' intravenous injection in the pre-clinical development.

Silica Nanotubes—Silica nanotube (SNT), a novel nanomaterial, has been developed for drug delivery,[194] biocatalysis,[195] and protein immobilization.[196] SNT has several advantages including large internal volume, large surface area, favorable biocompatibility, and the ability of being chemical modified.[197] More importantly, the size of SNT can be controlled easily.[198] Because of their unique physicochemical properties, SNT used as drug delivery system drew considerable attention from pharmaceutics researchers in recently. SNT possesses the potential as effective drug delivery vehicle owing to

1.   the large internal space of SNT can improve the drug loading capacity compared with other vectors. And the drug molecules incorporated in the inner of material cannot interact with the environment, so that the drug leakage was prevented until they reach the target destination;

2.   SNT possesses a plentiful of hydroxide radical on the surface which lead to the high hydrophilicity of nanotubes. The drug delivered by SNT can effectively avoid the capture by the RES, and improve the bioavailability of drug molecules;

3. SNT can be further modified to acquire more functions through the active hydroxide radicals on the surface. Bhattacharyya and coworker reported the sol–gel template synthesized SNTs that can be applied for controlled drug delivery in which ultrasound acts as the trigger.[194]

In order to control drug delivery, SNTs were coated by dihydroxynaphthalene (DN) and ibuprofen was chose as a model drug. The ibuprofen loading of SNTs and DN-SNTs analyzed by thermogravimetric analysis was 46% and 25%, respectively. The release kinetics of ibuprofen from SNTs demonstrated that the drug release is heavily dependent on the ultrasound impulse *in vitro*. Fluorescent silica nanotubes prepared via sol–gel reaction were also successfully applied for plasmid DNA delivery.[199] This work further demonstrated that SNTs have enormous potential in biomolecule drug delivery.

Gold

Among metal nanomaterials, those made of gold have been widely utilized in biomedical research. Gold nanomaterials have a variety of potential applications, including gene and drug delivery,[200] cell imaging,[201] thermotherapy,[202] and colorimetric detection of biomolecules and ions.[203–205] Nowadays, the synthesis and modification of gold nanomaterials can be effectively manipulated, and spheroids, rods, dodecahedra, octahedra, and cubes can be designed with a high level of precision.[206] The surface can also be modified with chemical functional groups, including amines, lipids, antibodies, peptides, oligonucleotides, and small molecules. In addition, the inherent non-toxicity and unique properties of gold nanomaterials, such as size- and shape-dependent cellular uptake and high surface area to volume ratio, make them excellent drug carriers.[207] Thus, considerable effort has been put in to develop gold nanomaterials as pharmaceutical adjuvants.

Gold Nanoparticles—Gold nanoparticles (Au NPs) have many attributes which meet the needs of nanomedicine research. Many works have focused on the modification of Au NPs with therapeutic agents and targeting ligands. Liang and co-workers recently synthesized novel small (2 nm) Au NPs that were functionalized with a therapeutic peptide, PMI (p12), and a targeting peptide, CRGDK, which selectively binds with cancer cells through the over-expressed receptor NRP-1.[208] The results demonstrated that these targeting nanocarriers improve the delivery of the therapeutic p12 peptide, which is thought to activate p53, into cancer cells. When employing Au NPs as drug carriers, conjugation is commonly used for drug loading and ligand modification. The problem is that available chemical groups on the surface are limited, so it is difficult to functionalize Au NPs with both therapeutics and targeting ligands. This work employed 2 nm Au NPs, the surface area to volume ratio of which is much higher than that of commonly used Au NPs, to provide more space for drugs and functional groups.

Gold Nanorods—The localized surface plasmon resonance (LSPR) effect is a widely utilized property of some special shaped gold nanomaterials, including nanorods, nanocages, nanoshells, and nanostars. Plasmon resonance occurs when gold nanomaterials are irradiated by light with specific wavelengths, and the luminous energy is converted into heat.[209] Direct killing of surrounding cells can be achieved by taking advantage of the heat released. This is called photothermal therapy. The heat released can also be further utilized to control the

behavior of nanomaterials by introducing thermosensitive materials into them.[210] Therefore, many gold nanomaterial-based light-controlled drug release systems have been developed based on the LSPR effect. Moreover, the absorption of light with specific wavelengths can be utilized for *in vivo* imaging (Fig. 9).

Among these gold nanostructures with LSPR effect, Au NRs are an outstanding one due to two reasons: (1) the uniformity and size of Au NRs are easier to control than that of other materials; (2) by manipulating the aspect ratio, the LSPR maximum of Au NRs can be tuned to the near-infrared (NIR) window (700–900 nm), which is the wavelength range in which lasers can penetrate deeply through tissues.[211] Wu and co-workers reported promising results in this area (Figs. 7(F), (G)).[212] They coated light-responsive Au NRs with mesoporous silica to overcome two major disadvantages of Au NRs, namely low specific surface area and poor stability. DOX was encapsulated into the mesoporous silica shell of the Au@SiO$_2$ nanoparticles. Low intensity laser irradiation triggered drug release, while high intensity laser irradiation also triggered hyperthermia. Furthermore, the Au@SiO$_2$ nanoparticles can be visualized by two-photon microscopy, so that imaging is also successfully integrated into this platform. The advantage of Au NRs is that they can be used as drug vehicle, photothermal agent, and two-photon microscopy probe. Thus, Au NRs are an inherent multifunctional drug delivery system. However, low loading capacity and template residues caused toxicity impede their application as pharmaceutical adjuvant. This work successfully solved both challenges at the same time by mesoporous silica coating, the unique properties of Au NRs were also maintained and utilized.

Photothermal therapy is a unique application of gold nanomaterials as they are not just drug vehicles, also therapy agents. The irradiation area, time, and intensity are tunable and the killing effect is direct. Future work should focus on the targeting ability of photothermal therapy agents as the therapy efficacy is determined by the biodistribution of heat source. Moreover, gold is inert in most conditions and its degradation and clearance remains unclear. As a result, the accumulation and excretion mechanism of gold nanomaterials in cells, organs, and bodies needs further study.

Other Gold Nanostructures—Other gold nanostructures, such as gold nanocages, gold nanoshells, and gold nanostars, are developed recently as photothermal therapy agent. Many work successfully combined photothermal therapy with imaging and drug delivery by employing these novel gold nanostructures.[213,214] For, example, a NIR laser-controlled drug release system which was based on smart polymers covered gold nanocages was successfully developed by Xia et al. The smart polymer undergoes a phase transition from a hydrophilic state to a hydrophobic state when the temperature is raised above its low critical solution temperature (LCST). Once covalently anchored to the surface of gold nanocages, the smart polymer would undergo the phase transition and aggregate under the heat converted by gold nanocages after NIR laser irradiation. The pre-loaded drug, lysozyme, can therefore be released in a controllable fashion using photothermal conversion of gold nanocages. Furthermore, gold nanocages have a potential for multi-functional drug delivery system after functionalized with targeting ligands. Hollow gold nanostructures are superior in drug loading as it would be easier than that of Au NRs and Au NPs as drug will diffuse into the hollow structure when incubating with these gold nanostructures and no chemical

reaction is needed. Moreover, hollow structure can provide more space compared to surface area. The biggest challenge is that the synthesis of these novel gold nanostructures is much more complicated than that of Au NRs and Au NPs. Thus, more efforts should be done to optimize the synthesis method and we believe that these gold nanostructures would become important members of gold-based nanoscale pharmaceutical adjuvant materials.

Other Inorganic Materials

Apart from silica and gold, other inorganic nanomaterials, such as carbon,[215] iron,[216–218] and calcium,[219] also have been used as pharmaceutical adjuvants. Some of these have special applications in pharmaceutical adjuvant research. For example, calcium phosphate nanoparticles can work as vaccine adjuvants, inducing immunity to viruses.[220] Results indicated that calcium phosphate nanoparticles were more potent as vaccine adjuvant than traditional aluminium adjuvant because less inflammation was caused and high titer of antibody was induced. Graphene oxide, a novel carbon nanomaterial with monolayer structure, is promising in aromatic drug delivery via non-covalently π–π stacking. SN38, a water-insoluble aromatic anticancer drug derived from camptothecin, is attached to graphene oxide by Dai and co-workers.[221] The obtained composite exhibited excellent water solubility while the anticancer efficacy was not compromised. Graphene oxide also showed potential in photothermal therapy, making it popular in novel medicine design.[222] Our previous work demonstrated that metallofullerene nanoparticles $Gd@C_{82}(OH)_{22}$, another promising carbon-based nanomaterial, can circumvent tumor resistance to cisplatin by reactivating endocytosis, which is a new strategy to fight with tumor drug resistance.[223] Further studies demonstrated that $Gd@C_{82}(OH)_{22}$ nanoparticles are inherently anticancer drug.[224,225] They can inhibit pancreatic tumor metastasis by affecting matrix metalloproteinase-9 (MMP-9) function. Another potential pharmaceutical adjuvant material is superparamagnetic iron oxide nanoparticles (SPIONs) which are well-known as contrast agents.[226] Thus, SPIONs are appropriate for design of traceable drug delivery system. Tumor site accumulated SPIONs will enable to monitor the growth, metastasis, and location of tumor tissue. Following these established examples, inorganic nanomaterials can be used selectively to design drug vehicles with more functions.

HYBRID ORGANIC AND INORGANIC NANOMATERIALS FOR PHARMACEUTICAL APPLICATION

Multifunctional nanoparticles, which are desired for advanced drug delivery, can be created by incorporating more than one nanomaterial into a nanostructure. These combined nanomaterials are known as hybrid nanomaterials, and they combine the advantages of each component. In another way, fatal drawbacks of one component are made up by the other one. Recently, thermal and pH dual responsive hybrid nanoparticle based on this strategy was successfully developed (Fig. 7(H)).[227] The hybrid nanoparticles had a SiO_2 shell and a polymer core made of poly(N-isopropylacrylamide) (PNiPAM)-co–acrylic acid (AA) hydrogel, which is responsive to both heat and pH. By lowering the temperature, DOX was loaded into the nanoparticles and by manipulating the pH, DOX was rapidly released. Compared with traditional MSNs, these hybrid nanoparticles had a better uniformity and stability. Moreover, the *in vitro* results showed that these novel nanoparticles were more bio-

compatible and less toxic than conventional MSNs, while the anticancer efficiency was not compromised. In this work, SiO_2 is employed as the drug container, but pH-responsive drug release could not be realized in pure silica system. As a result, pH-sensitive polymer is introduced as the core of the nanoparticle. This core–shell drug delivery system works like a tank: SiO_2 shell is the armor, DOX is the cannonball, and PNiPAM/AA hydrogel is the fire control system. It cruises everywhere, resists attack from clearance system, and fires in the battle-field (slightly altered acidic tumor microenvironment and endosomes).

Theranostic, a new concept combins therapy and diagnostic, is emerging with the development of hybrid adjuvants.[228,229] It is difficult to realize theranostic with only one adjuvant material. Therefore, hybrid adjuvants composed of more than one material are employed. For instance, after the drug-loaded nanoparticles are administered for therapeutic treatment, biodistribution of these nanoparticles is expected to be monitored in organs and disease site. In this case, fluorescence probes or MRI contrast agents can be integrated into the drug vehicle. The labeled hybrid nanoparticles are then monitored non-invasively throughout the treatment and researchers can get more information from a single injection.

Actually, most examples we mentioned in this article are hybrid materials but only one component is discussed in detail in individual context. It is a trend to combine more functions in one nanoparticle, resulting in the widely application of hybrid nanomaterials. Although it is fascinating to design multifunctional drug delivery system with hybrid nanomaterials, there are two disadvantages under consider in application. First, the preparation will become complicated compared to that of single-component nanoparticles, resulting in the reduction of reproducibility. Second, the drug release pathway and metabolism mechanism will be unclear after the addition of another material. Both of them are of great importance for pharmaceutical adjuvants. Therefore, every aspect of hybrid materials should be thoroughly studied.

CONCLUSIONS

Recent progress reveals the great potential of nanoscale materials as novel pharmaceutical adjuvants. Nanomaterials are capable of transporting insoluble drugs in aqueous solution, reducing adverse side-effects, delivering dual therapeutic agents simultaneously, targeting drugs to disease lesions and controlling drug release in the desired environment. However, despite the enormous potential of nanoscale materials, considerable challenges must be addressed before they can be translated into clinical use. Pharmaceutical adjuvants must fulfill specific criteria, like biocompatibility, stability in physicochemical conditions, protection of active drugs, and low toxicity, in order to meet the requirements of the FDA. In addition, these materials should be reproducible in a scalable manner with high purity and low cost. Most reported nanomaterials are synthesized and modified in small batches. Standard protocols must be provided for industrial-scale production of nanoscale pharmaceutical adjuvant materials. Comprehensive assessment of the biosafety issues of nanomaterials, such as biocompatibility, biodegradation and biodistribution, should also be provided. Drug delivery mechanisms should be further studied for a better understanding of pharmacodynamics and pharmacokinetics of different nanocarriers. As far as we know, size, shape, charge, functional groups, and other propeties of nanomaterials all have an impact on

their interaction with biosystems. We need to understand the role played by each property, so that the pharmacological attributes of nanomaterials can be optimized. Nowadays, many scientists are working hard to overcome these challenges and we are sure that nanoscale materials have a bright future as pharmaceutical adjuvants.

Acknowledgments

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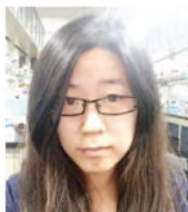
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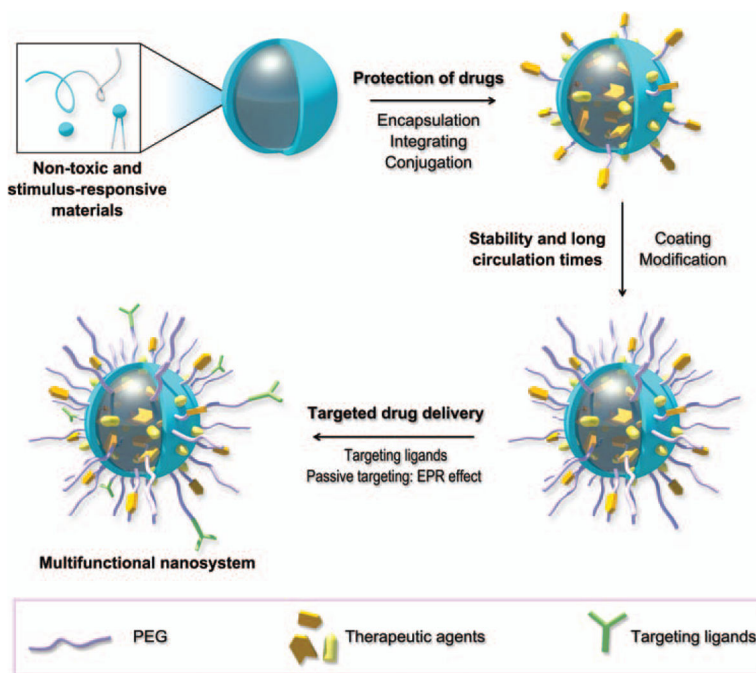
Figure 1.
The desired characteristics of a multifunctional nanosystem composed of nanoscale pharmaceutical adjuvant materials. (A) The materials should be non-toxic to satisfy biosafety criteria and responsive to stimuli for triggered release of drugs. (B) Drugs should be carefully protected before they reach the disease site. (C) The drug vehicle should be stable in the physiological environment and resistant to clearance. (D) By conjugating with targeting ligands, active targeting can be achieved. Meanwhile, passive targeting can be realized by controlling the size of the multifunctional nanosystem.
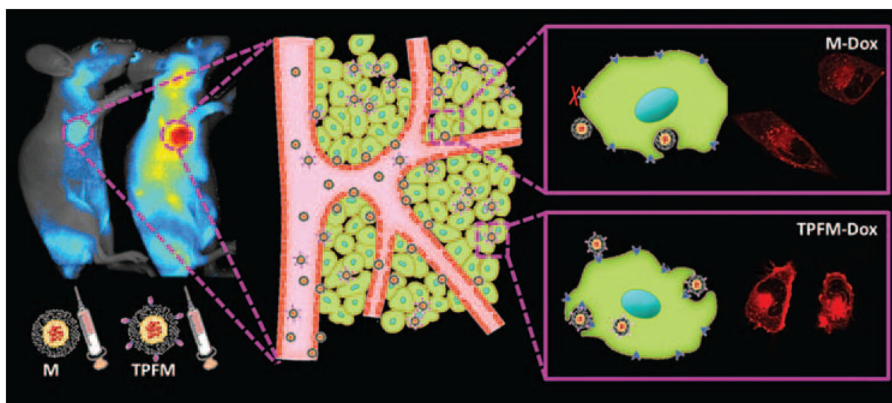
Figure 2.
Passive and active targeting of nanoparticles. M-Dox: non-functionalized Dox-encapsulated micelle; TPFM-Dox: tumor-penetrating peptide functionalized Dox-encapsulated micelle. The shade of color in the images stands for the amount of nanoparticles in tumors and in cells. Reprinted with permission from [24], T. Wei, et al., Functionalized nanoscale micelles improve drug delivery for cancer therapy *in vitro* and *in vivo*. *Nano Lett.* 13, 2528 (2013). © 2013, American Chemical Society.
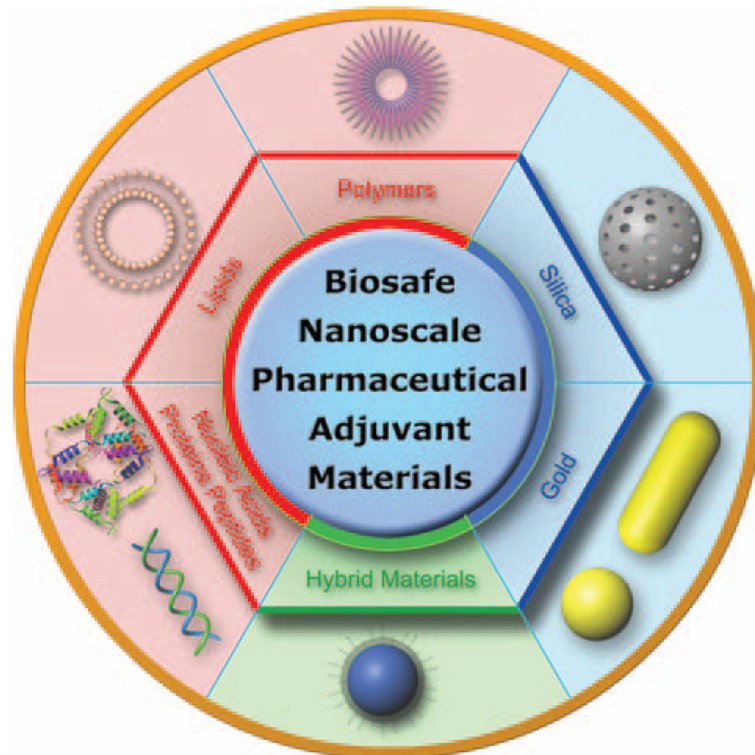
Figure 3.
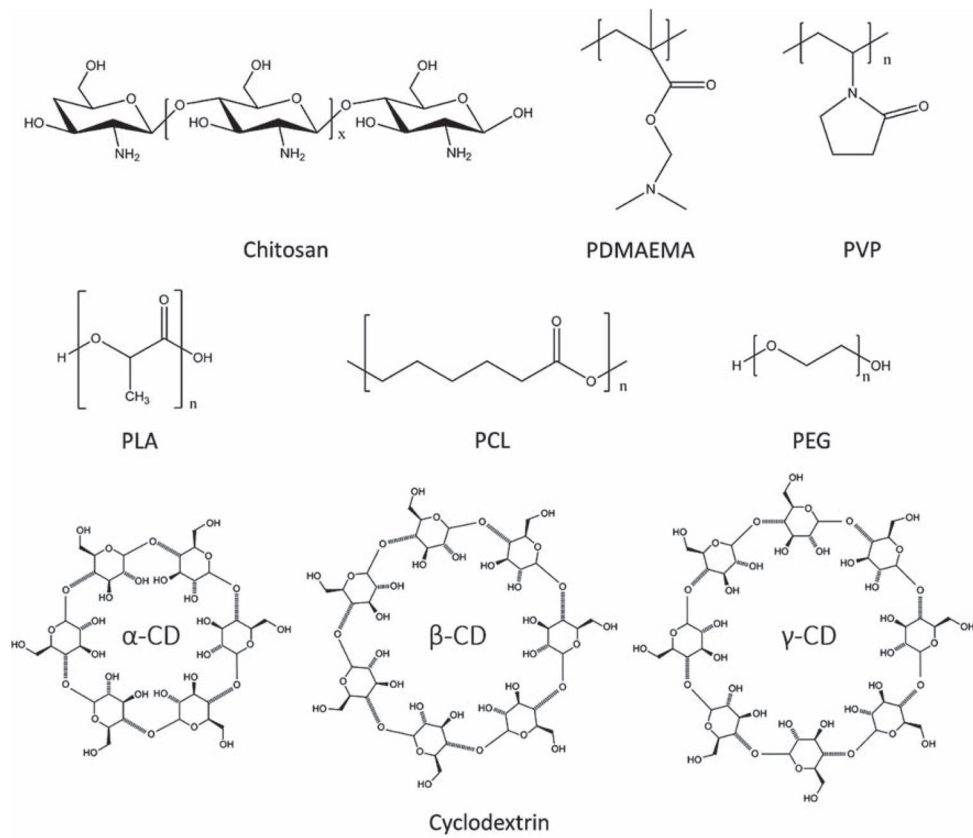Representative biosafe nanoscale pharmaceutical adjuvant materials.

Figure 4.
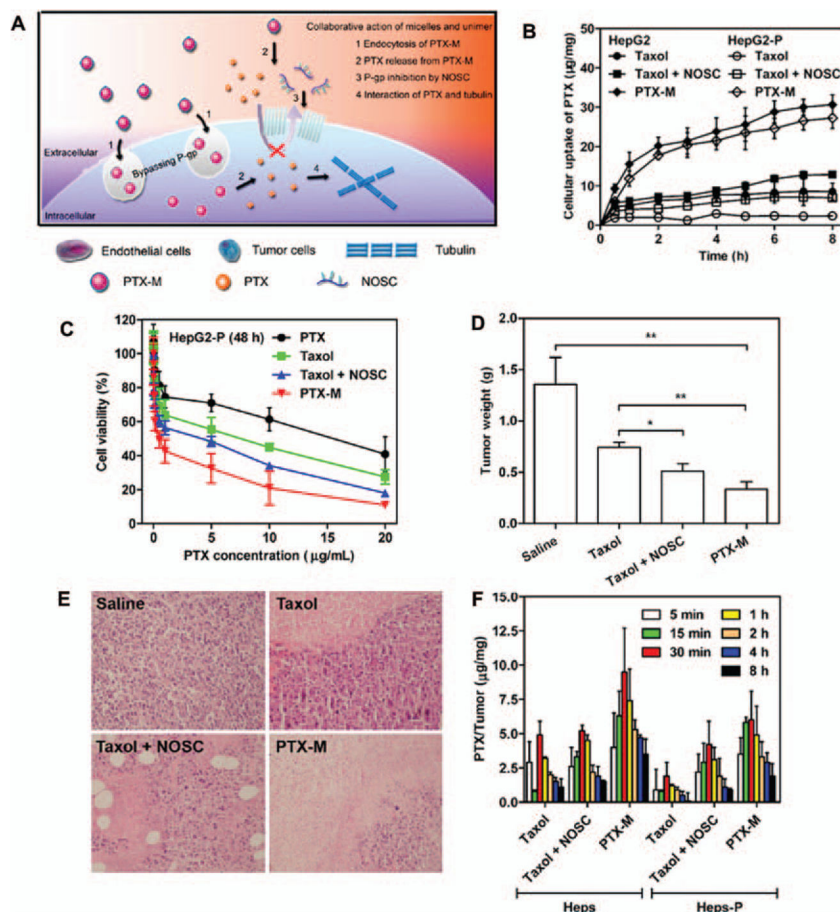Chemical structures of representative polymers and cyclodextrins as pharmaceutical adjuvant materials.

Figure 5.
A chitosan-based micelle that can overcome drug resistance of HepG2 cells. (A) Scheme of P-gp inhibition of NOSC and P-gp bypassing of PTX. (B) Cellular uptake of different PTX formulations in HepG2 and HepG2-P cells. (C) Cytotoxicity evaluation of different PTX formulations toward HepG2-P cells after 48 h treatment. (D) Tumor weights of HepG2-P xenograft mice model after different PTX formulation treatment at day 21. (E) H&E stained tumor sections after the treatment at day 21. (F) PTX accumulation in tumor tissue after intravenous injection of different PTX formulations into Heps and Heps-P tumor-bearing mice. Reprinted with permission from [77], X. Jin, et al., Paclitaxel-loaded *N*-octyl-*O*-sulfate chitosan micelles for superior cancer therapeutic efficacy and overcoming drug resistance. *Mol. Pharm.* 11, 145 (2014). © 2014, American Chemical Society.
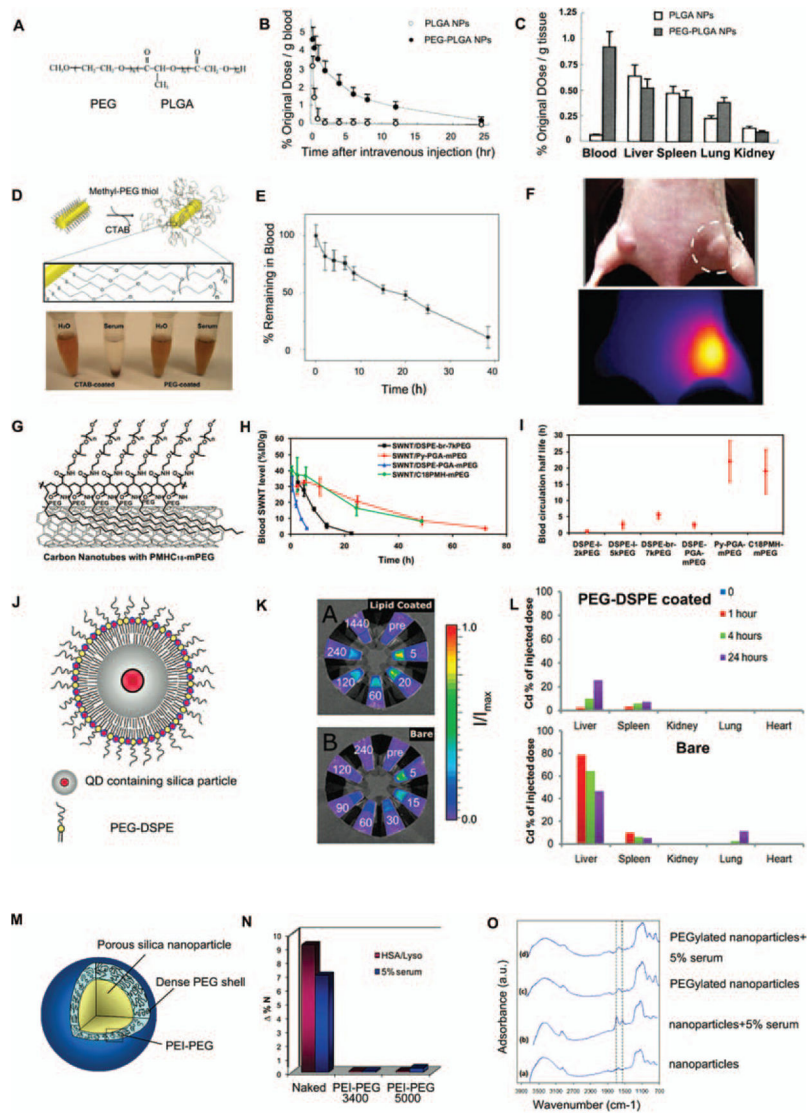
Figure 6.
PEG-protected nanoparticles with improved pharmacokinetics. (A) Structure of the PEG–PLGA copolymer. (B) Blood clearance curves of I[125]-labeled BSA in PEG-PLGA and PLGA nanoparticles. (C) Tissue distribution of I[125]-labeled BSA at 12 h in PEG-PLGA and PLGA nanoparticles. Reprinted with permission from [89], Y.-P. Li, et al., PEGylated PLGA nanoparticles as protein carriers: Synthesis, preparation and biodistribution in rats. *J. Control Release* 71, 203 (2001). © 2001, Elsevier. (D) Molecular schematic of PEG coating on NR surface and stability of NRs in water and serum. (E) Blood clearance curves of PEG-protected Au NRs. (F) Thermal imaging of PEG-protected Au NRs in tumor tissue 72 h post intravenous injection. Reprinted with permission from [90], G. von Maltzahn, et al., Computationally guided photothermal tumor therapy using long-circulating gold nanorod antennas. *Cancer Res.* 69, 3892 (2009). © 2009, American Association for Cancer Research. (G) SWNTs coated with PMHC18-mPEG. (H) Blood circulation curves of different PEG-coated SWNTs. (I) Blood circulation half-lives of different PEG-coated SWNTs. Reprinted

with permission from [91], G. Prencipe, et al., PEG branched polymer for functionalization of nanomaterials with ultra-long blood circulation. *J. Am. Chem. Soc.* 131, 4783 (2009). © 2009, American Chemical Society. (J) Schematic representation of PEG-protected QD containing silica particle. (K) Fluorescence intensity of blood samples taken at different time points (indicated in minutes). (L) Biodistribution analysis of bare and PEG-protected particle by cadmium (Cd, a component of the QD) quantification. Reprinted with permission from [92], M. M. van Schooneveld, et al., Improved biocompatibility and pharmacokinetics of silica nanoparticles by means of a lipid coating: A multimodality investigation. *Nano Lett.* 8, 2517 (2008). © 2008, American Chemical Society. (M) Schematic structure of the PEGylated silica nanoparticle. (N) Nonspecific protein adsorption of protein on naked and PEG-protected silica nanoparticles after incubation with protein solution. (O) FTIR spectra of naked and PEG-protected silica nanoparticles after incubation with 5% serum solution. Reprinted with permission from [93], B. Thierry, et al., Electrostatic self-assembly of PEG copolymers onto porous silica nanoparticles. *Langmuir* 24, 8143 (2008). © 2008, American Chemical Society.
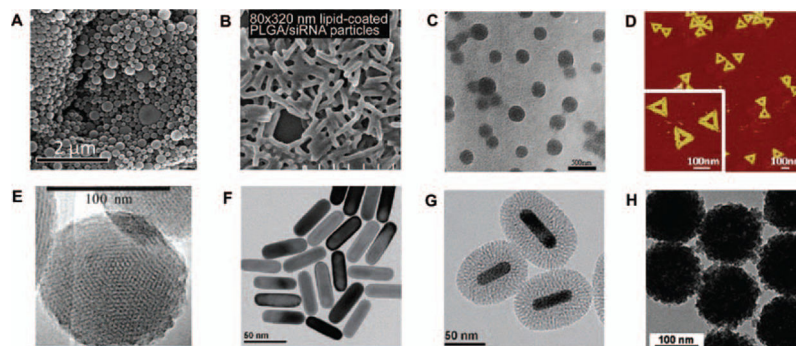
Figure 7.
Microscope images of (A) acetal-derivatized dextran nanoparticles. Reprinted with permission from [105], E. M. Bachelder, et al., Acetal-derivatized dextran: An acid-responsive biodegradable material for therapeutic applications. *J. Am. Chem. Soc.* 130, 10494 (2008). © 2008, American Chemical Society. (B) lipid-coated PLGA/siRNA particles. Reprinted with permission from [130], W. Hasan, et al., Delivery of multiple siRNAs using lipid-coated PLGA nanoparticles for treatment of prostate cancer. *Nano Lett.* 12, 287 (2011). © 2011, American Chemical Society. (C) cisplatin-loaded milk protein nanoparticles. Reprinted with permission from [148], X. Zhen, et al., Cellular uptake, antitumor response and tumor penetration of cisplatin-loaded milk protein nanoparticles. *Biomaterials* 34, 1372 (2012). © 2012, Elsevier. (D) DNA origami nanostructures. Reprinted with permission from [173], Q. Jiang, et al., DNA origami as a carrier for circumvention of drug resistance. *J. Am. Chem. Soc.* 134, 13396 (2012). © 2012, American Chemical Society. (E) MSNs. Reprinted with permission from [191], I. Slowing, et al., Effect of surface functionalization of MCM-41-type mesoporous silica nanoparticles on the endocytosis by human cancer cells. *J. Am. Chem. Soc.* 128, 14792 (2006). © 2006, American Chemical Society. (F) Au NRs; (G) Au@SiO$_2$ nanoparticles. Reprinted with permission from [212], Z. Zhang, et al., Mesoporous silica-coated gold nanorods as a light-mediated multifunctional theranostic platform for cancer treatment. *Adv. Mater.* 24, 1418 (2012). © 2012, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. (H) PNiPAM/AA@SiO$_2$ nanoparticles. Reprinted with permission from [227], X. Hu, et al., Multifunctional hybrid silica nanoparticles for controlled doxorubicin loading and release with thermal and pH dual response. *J. Mater. Chem. B* 1, 1109 (2013). © 2013, Royal Society of Chemistry.
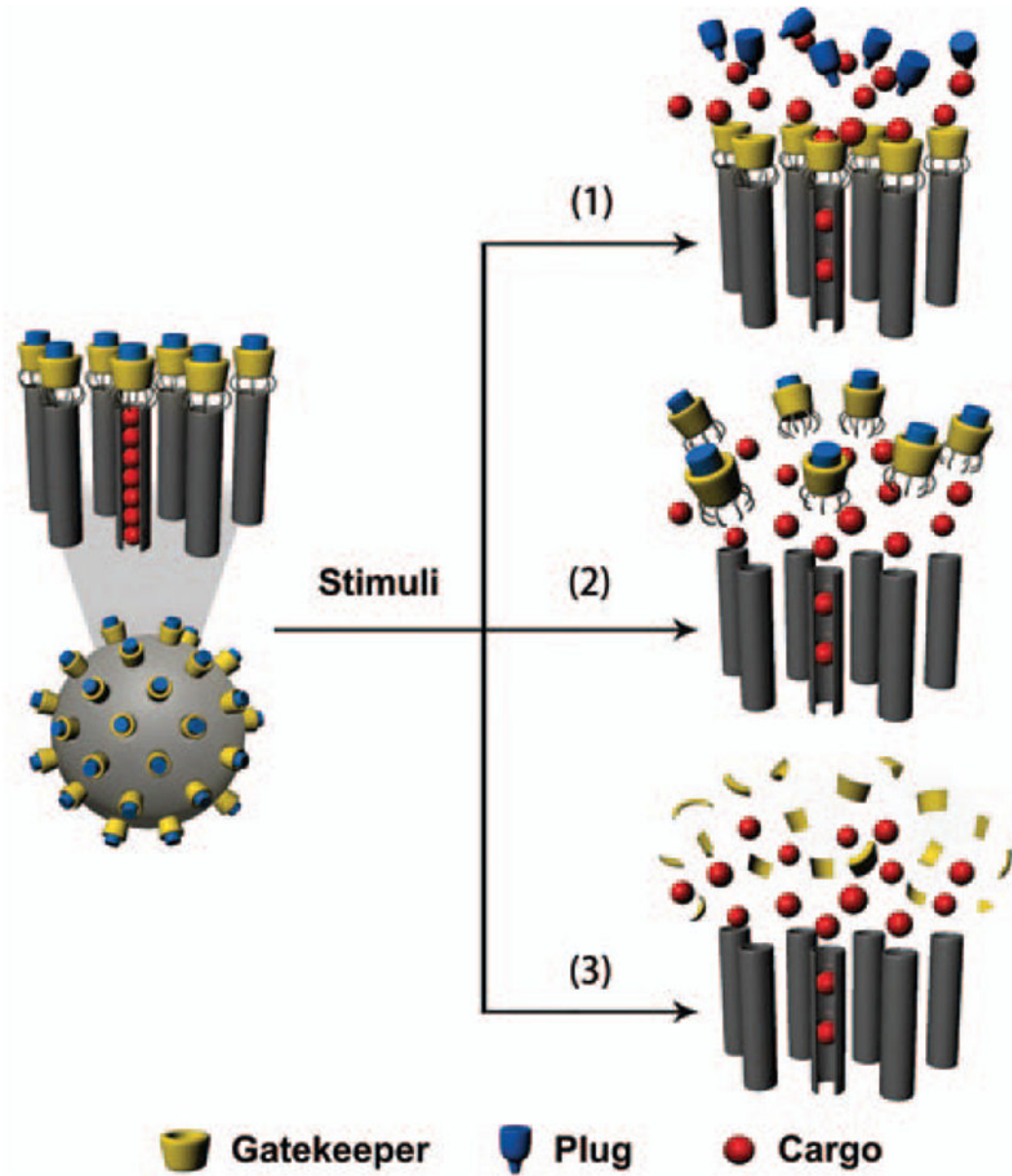
Figure 8.
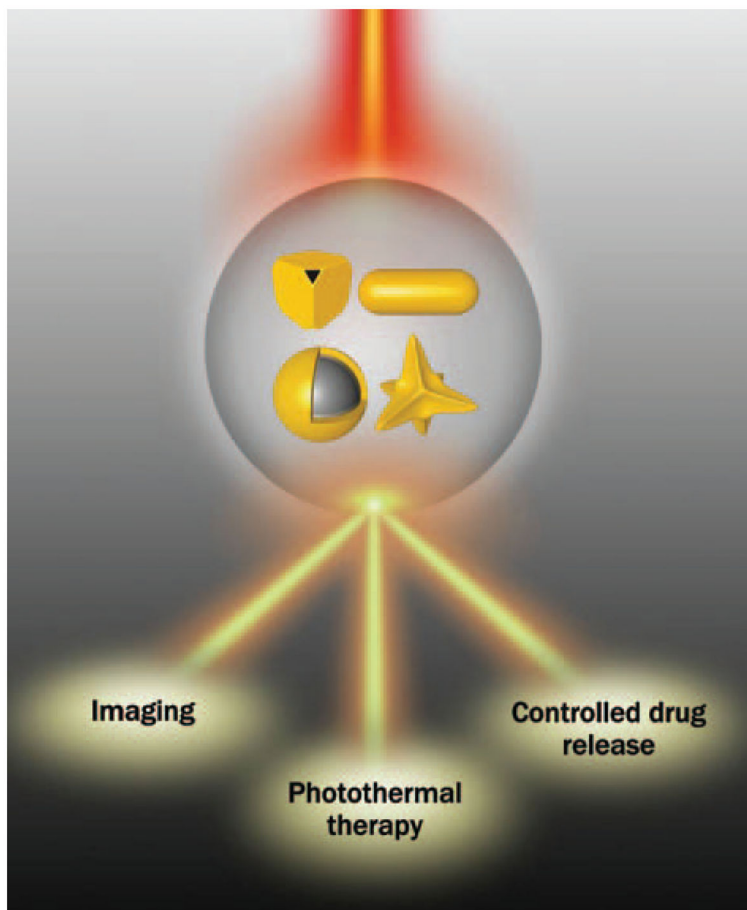Schematic illustration of three different nanovalve-based stimuli-responsive controlled drug release mechanisms in MSNs.

Figure 9.
Applications of LSPR effect of gold nanostructures in pharmaceutical research.

Table I

Structures of commonly used lipids in pharmacy research.

Lipid	Abbr.	Structure
L-α-phosphatidylcholine, hydrogenated (Soy)	HSPC	
1,2-dioctadecanoyl-sn-glycero-3-phosphoethanolamine	DSPE	
1,2-dioctanoyl-sn-glycerol	DG	
L-α-phosphatidic acid	PA	
L-α-phosphatidylglycerol	PG	
1,2-dilauroyl-sn-glycero-3-ethylphosphocholine	Ethyl PC	
1,2-di-O-octadecenyl 3-trimethylammonium propane	DOTMA	
Cholesterol	Chol.	

Table II

Examples of targeting ligands.

Category	Name	Target and indication	Refs.
Protein			
Antibody	Trastuzumab (Herceptin®)	HER2/neu	[230, 231]
	Bevacizumab (Avastin®)	VEGF	[232]
	Cetuximab (Erbitux®)	EGF receptor	[233, 234]
	Rituximab (Rituxan®)	CD20	[235]
	Ibritumomab tiuxetan (Zevalin®)	CD20	[236]
	Tositumomab and tositumomab (Bexxar®)	CD20	[237]
	Gemtuzumab ozogamicin (Mylotarg®)	CD33	[238]
	Alemtuzumab (Campath®)	CD52	[239]
Others	Transferrin	Transferrin receptor	[240, 241]
	Low density lipoprotein	Folate receptor	[242]
	Heavy-chain ferritin	Transferrin receptor 1	[243]
Peptide	RGD	$\alpha v\beta 3$-integrin	[244, 245]
	CRGDK	Neuropilin-1	[24]
	NGR	CD13	[246]
	SP5-52	Tumor neovasculature	[247]
	PIVO-8	Tumor angiogenesis	[248]
	PIVO-24	Tumor angiogenesis	[248]
	LyP-1	Tumor hypoxia and lymphangiogenesis	[249]
	RVG	Acetylcholine receptor	[250]
	TAT	Cell nucleus	[166, 251]
Nucleic acid: aptamer	Macugen	Vascular endothelial growth factor	[252]
	AS1411	Nucleolin	[253, 254]
	sgc8	Protein tyrosine Kinase 7	[255]
	S1.3/S2.2	Mucin 1	[256]
	TD05	Immunoglobulin μ-heavy chains	[255]
	A10	Prostate-specific membrane antigen	[257]
	TBA	α-thrombin	[258]
	Apt-$\alpha v\beta 3$	$\alpha v\beta 3$ integrin	[259]
	B28	HIV gp120	[260]
	A30	HER3	[261]