Current Topics

Recent Progress in Drug Delivery System for Cancer Therapy

Review

Co-delivery Systems of Multiple Drugs Using Nanotechnology for Future Cancer Therapy

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Cancer treatments have improved significantly during the last decade but are not yet satisfactory. Combination therapy is often administered to improve efficacy and safety. Drug delivery systems can also improve efficacy and safety. To control the spatiotemporal distribution of drugs, nanotechnology involving liposomes, solid lipid nanoparticles, and polymeric micelles has been developed. Co-delivery systems of multiple drugs are a promising approach to combat cancer. Synergistic effects and reduced side effects are expected from the use of co-delivery systems. In this review, we summarize various co-delivery systems for multiple drugs, including small-molecule drugs, nucleic acids, genes, and proteins. Co-delivery of drugs with different properties is relatively difficult, but some researchers have succeeded in developing such co-delivery systems. Environment-responsive carrier designs can control the release of cargos. Although their preparation is more complicated than that of mono-delivery systems, co-delivery systems can simplify clinical procedures and improve patient QOL.

Key words liposome; polymer; small-molecule drug; DNA; RNA; protein

1. Introduction

Cancer is highly lethal and accounts for approximately 26% of overall deaths worldwide.[1] The 5-year net survival rate varies depending on the type of cancer. For example, the 5-year survival rate of prostate cancer patients is high (in the range of 70–100%), whereas that of pancreatic cancer patients remains low (5–15%).[2] In general, treatment outcomes of resectable cancer are satisfactory, but the prognoses of patients with advanced, unresectable cancer are poor. Chemotherapy has been developed to treat unresectable cancer,[3,4] and molecular targeting drugs such as tyrosine kinase inhibitors and therapeutic antibodies have improved both efficacy and safety.[5–8] Recently, marked progress has been made in cancer immunotherapies such as immune checkpoint inhibitors and chimeric antigen receptor-T cell (CAR-T cell) therapy.[9–11] Although cancer treatment has improved significantly during the last decade, there is still room for improvement in patient outcomes.

Combination cancer therapy is superior to monotherapy in terms of efficacy and safety.[12,13] For example, the combination of 5-fluorouracil (5-FU), levofolinate, irinotecan, and oxaliplatin (FOLFIRINOX)[14] or of gemcitabine and nab-paclitaxel[15] is first-line therapy for advanced and unresectable pancreatic cancer and superior to gemcitabine monotherapy. Postoperative and preoperative combination chemotherapies are also administered as adjuvant and neoadjuvant therapy.[16] In cancer immunotherapy, the combination of chemotherapy with an immune checkpoint inhibitor[17] or CAR-T cells[18,19] improves patient outcomes because it induces immunogenic cell death.

Drug delivery systems (DDS), defined as systems enabling spatiotemporal control of drug distribution in the body to improve efficacy and safety, have been developed for cancer treatment. Several formulations have been clinically approved, *e.g.*, PEGylated liposomal doxorubicin (Doxil or Caelyx injection) and albumin-bound paclitaxel (nab-paclitaxel, Abraxane as an injectable suspension).[20] To control spatiotemporal drug distribution, nano-DDS carriers such as liposomes,[21,22] solid lipid nanoparticles,[23] polymeric micelles,[24] nanogels,[25] and polymer nanoparticles[26] have been used. Targeting of specific organs and cells is also a promising approach to cancer treatment.[27,28] Recently, progress has been made in stimuli-responsive smart DDS,[29] particularly in those combining physical stimuli with nanoparticles delivered to specific regions in target tissues.[30] DDS for combination therapy would further improve efficacy and safety.[31–33] The co-delivery of multiple drugs to the same (cancer) cells is expected to yield not only additive but also synergistic effects. This review summarizes co-delivery systems of multiple drugs, especially those with different properties such as lipophilicity and electrostatic charge (Fig. 1).

2. Delivery Carriers for Co-delivery

Liposomes are major carriers in the DDS field which can deliver both hydrophilic and lipophilic drugs.[34] Hydrophilic drugs are carried in the inner space of liposomes, while lipophilic drugs are partitioned to the lipid bilayer. Liposomes can thus theoretically co-deliver multiple drugs with different lipophilicity. In addition, cationic liposomes form complexes (lipoplexes) with nucleic acids and plasmid DNA,[35,36] making liposomes extremely versatile. Polymers including polymeric micelles can deliver lipophilic drugs,[37] nucleic acids,[38] and

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genes.[39] Hydrophilic drugs can be conjugated with polymers as polymeric prodrugs.[40]

Drug release is an important issue because drugs are generally inactive before dissociation from carriers. The utilization of environment-responsive mechanisms[41–43] and supramolecular chemistry such as polyrotaxanes are useful strategies to control drug release.[44,45] Nanogels are promising carriers that deliver both lipophilic and hydrophilic drugs,[46,47] and nucleic acids.[48] The release of drugs from nanogels can be controlled by various environmental changes, including pH, temperature, redox state, and enzymes.[49] However, the release profiles of multiple drugs from single carriers may differ due to the different drug properties. Synchronized biodistribution and release profiles of multiple drugs are preferable to achieve synergistic effects. It may be possible to select carriers depending on the cargo properties. The requirements for carriers in co-delivery systems are summarized in Fig. 2.

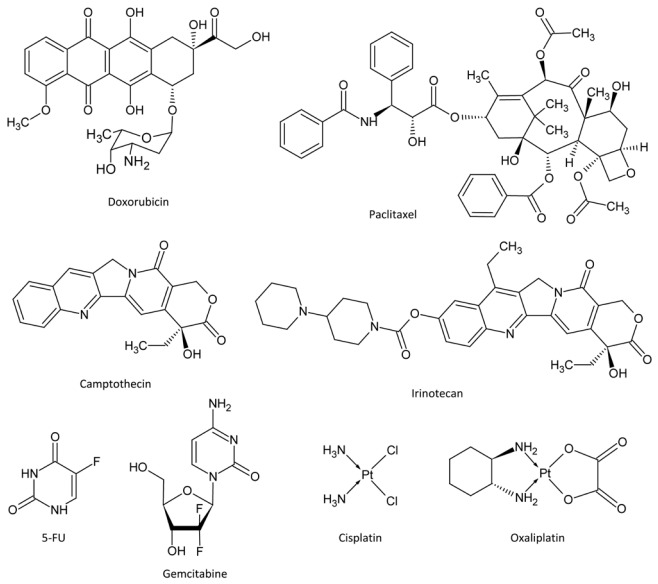

Fig. 1. Chemical Structures of Anticancer Drugs for Co-delivery Systems

3. Co-delivery of Multiple Small-Molecule Drugs

Among the various combinations of anticancer drugs, 5-FU and oxaliplatin combined with folinic acid (a 5-FU enhancer), known as FOLFOX, are commonly used as adjuvant chemotherapy for colorectal cancer and stage IV recurrent colorectal cancer.[50,51] In combination therapy, multiple drugs with different mechanisms of action are used to increase efficacy and decrease the severity of side effects. An appropriate co-delivery strategy is essential in combination therapy, but the dosage schedule (regimen) for co-delivery systems is simpler than that for combination therapy. Therefore, not only can efficacy and safety be improved, but the patient burden can also be re-

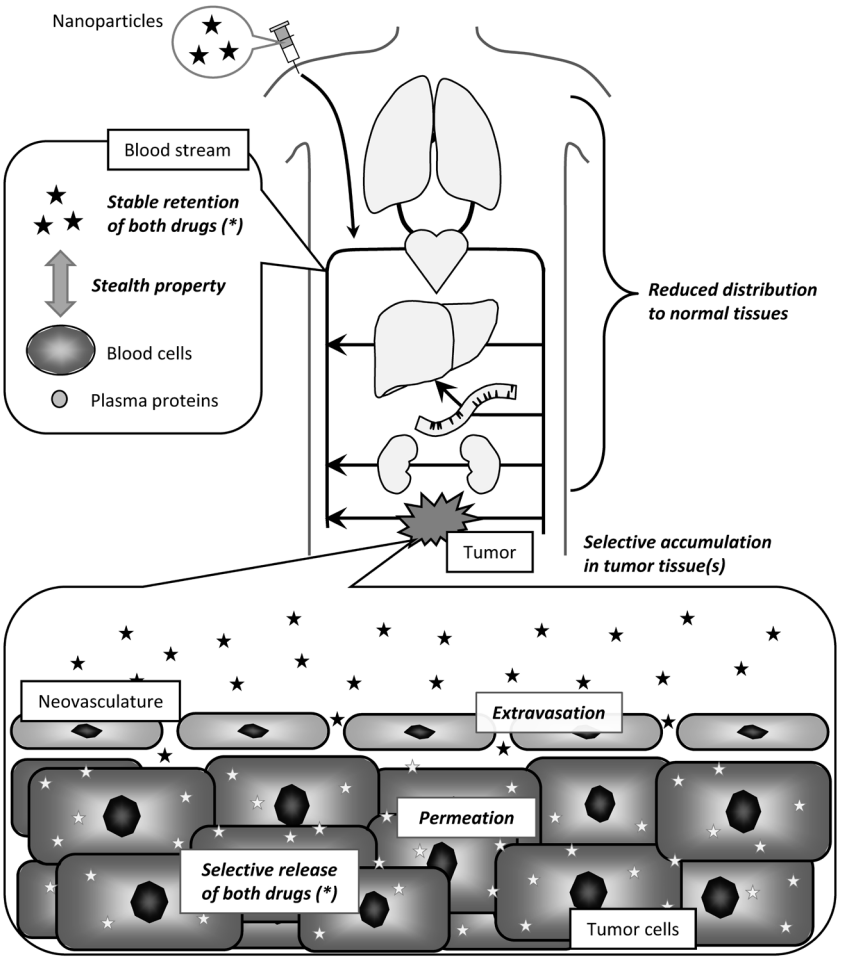

Fig. 2. Requirements for Tumor-Targeting Co-delivery Systems

Specific points for co-delivery systems are indicated by asterisks (*).

duced with the use of co-delivery systems. Although combinations in co-delivery systems generally correspond to the multiple agents used in combination chemotherapy, there is also the potential for unique combinations. In many cases, at least one potent drug is chosen as an element of the combination. The other elements are chosen from among potent agents with different mechanisms and drugs or natural products contributing to the efficacy of chemotherapy. Doxorubicin, paclitaxel, camptothecin, and 5-FU are often chosen as anticancer drugs, and common combination groups are shown in Table 1.

Doxorubicin is an inhibitor of topoisomerase II, RNA polymerase, *etc.* and induces apoptosis.[52] Doxorubicin alone is effective in treating malignant lymphoma, lung cancer, gastrointestinal cancer (gastric cancer, bile duct cancer, liver cancer, colorectal cancer, *etc.*), breast cancer, bladder tumors, and osteosarcoma. Doxorubicin is sparingly water soluble, and its partition coefficient ($\log P$ value) is 1.27.[53] Several groups have developed co-delivery systems of doxorubicin with camptothecin or its derivatives. Camptothecin is a lipophilic inhibitor of topoisomerase I. Tai *et al.* synthesized a PEGylated polymeric prodrug of camptothecin[54] which forms nanoparticles with doxorubicin in water due to the hydrophobicity of the camptothecin moiety, similar to polymeric micelles. The nanoparticle diameter was about 50 nm, which was suitable for passive targeting *via* the enhanced permeability and retention (EPR) effect.[55] In a lung tumor xenograft model, the co-delivery system exerted anticancer effects superior to those of the polymeric prodrug alone, indicating the usefulness of co-delivery.

Gao *et al.* reported a similar co-delivery system, which was a PEGylated camptothecin polymeric prodrug with a reduction- and pH-responsive design.[56] That polymeric prodrug formed polymeric micelles with diameters of approximately 70 nm with doxorubicin. The redox-responsive release of camptothecin and pH-responsive release of doxorubicin were demonstrated. Among other camptothecin derivatives, Zhang *et al.* synthesized an amphiphilic, pH-responsive PEGylated doxorubicin prodrug that formed nanoparticles with hydrophobic 10-hydroxycamptothecin.[57] That co-delivery system suppressed cancer cell growth more efficiently than the physical mixture of the doxorubicin prodrug and 10-hydroxycamptothecin, indicating the advantages of co-delivery. Wang *et al.* developed a water-in-oil-in-water (w-o-w) emulsion of doxorubicin and irinotecan using polylactide-*co*-glycolide (PLGA), the detergent Pluronic F127, hyaluronic acid, and chitosan-Pluronic F127.[58] Irinotecan is a prodrug of the active metabolite SN-38, a camptothecin derivative, and irinotecan was encapsulated in the oil core of the emulsion.

Hyaluronic acid is often utilized as a moiety targeting CD44, which is overexpressed in cancer stem-like cells. Although drug loading increased particle size, the diameters of nanoparticles containing both doxorubicin and irinotecan were less than 70 nm. This system exhibited strong antitumor effects in a human breast tumor model. As examples of other combinations, Han *et al.* developed amphiphilic dendrimer-based nanomicelles about 70 nm in diameter to deliver doxorubicin and 5-FU.[59] Chen's group formulated 100–160-nm pH-sensitive liposomes co-loaded with doxorubicin and the molecular-targeting drug imatinib with folate moieties.[60] He *et al.* developed a mesoporous silica–liposome hybrid system carrying doxorubicin and the molecular-targeting drug erlotinib.[61] The diameter of the silica core was 80 nm, and it was coated with an approximately 8-nm lipid layer. Cryotransmission electron microscopic images showed that complexes consisting of several particles were formed. Lakkadwala *et al.* investigated transferrin and penetratin dual-functionalized liposomes with diameters of 180 nm encapsulating doxorubicin and erlotinib.[62]

Not only anticancer drugs but also natural products have been used as co-delivery partners. Among them, curcumin is an attractive natural product to improve chemosensitivity, chemoresistance, and chemoprotection.[63,64] Curcumin is a lipophilic compound with a $\log P$ value of 3.[65] Zhang *et al.* synthesized a pH-sensitive amphiphilic polymer that formed nanoparticles 100 nm in diameter containing doxorubicin and curcumin.[66] We also developed polymer-assisted PEGylated lipid-calcium carbonate nanoparticles encapsulating doxorubicin and curcumin with diameters of 100 nm.[67] Chen *et al.* synthesized a cyclodextrin-based amphiphilic polymer allowing ratiometric mixing of doxorubicin, camptothecin, and curcumin.[68] That polymer formed relatively large 150-nm micelles. On the other hand, Tao's group synthesized an amphiphilic polymer to carry doxorubicin and the lipophilic drug disulfiram,[69] which is used to treat alcohol abuse. The anticancer effects of disulfiram are not dependent on acetaldehyde dehydrogenase inhibition but rather target nuclear protein localization 4 (NPL4).[70,71] Particle sizes are dependent on the nature of doxorubicin partners and range from 50 nm to 180 nm.

Paclitaxel is a highly lipophilic ($\log P$ value = 7.4) stabilizer of microtubules and inhibitor of cell division.[72] Injectable Taxol, the clinically available form of paclitaxel, is an ethanol solution containing the nonionic detergent polyoxyethylated castor oil. Therefore, co-delivery systems that can encapsulate both hydrophilic and lipophilic drugs are candidates for combination with paclitaxel. Wang *et al.* used double (w/o/w) emulsions with PEG–PLGA to encapsulate paclitaxel and doxorubicin.[73] The particle size of the double emulsions was greater than 200 nm and therefore the particles were relatively large. Zhu *et al.* developed folate-modified polymersomes using a polycaprolactone (PCL)–PEG–PCL triblock copolymer, which also had a particle size greater than 200 nm.[74] Alginate/calcium carbonate nanoparticles containing paclitaxel and doxorubicin developed by Wu *et al.* had a diameter of 150 nm.[75] Therefore, it seems difficult to reduce the size of these nanoparticles to less than 100 nm for the combination of paclitaxel and doxorubicin.

Since the gemcitabine and nab-paclitaxel combination is currently first-line therapy for advanced and unresectable pancreatic cancers, this combination is reasonable for co-delivery. However, gemcitabine is more water soluble than doxorubicin, and thus it may be more difficult to encapsulate both paclitaxel and gemcitabine into single particle. Meng *et al.* successfully encapsulated paclitaxel and gemcitabine using mesoporous silica nanoparticles coated with lipids,[76] in which gemcitabine was partitioned in mesoporous silica nanoparticles, and paclitaxel was partitioned in the lipid layer. The resulting particle size was 75 nm. Zhang *et al.* entrapped paclitaxel and gemcitabine into lipid/calcium phosphate (LCP),[77] which was originally developed by Huang's group.[78,79] In LCP, gemcitabine was entrapped in the calcium phosphate core, and paclitaxel was partitioned in the lipid layer. The par-

Table 1. Co-delivery Systems Containing Small-Molecule Drugs

Cargos	Carriers	Particle size	Release mechanisms	Evaluations	Ref.
Dox, CPT polymeric prodrug	PEGylated *graft* co-polymer	30–65 nm	Physical degradation (Dox), Esterase (CPT)	*In vitro* release, *in vitro* uptake, *in vivo* biodistribution, *in vivo* anti-tumor efficacy	54
Dox polymeric prodrug, CPT polymeric prodrug	Polymer (POEGMA) with β-cyclodextrin	70 nm	pH (Dox), Redox (CPT)	*In vitro* release, cell viability, *in vitro* uptake, blood compatibility	56
Dox polymeric prodrug, HCPT	PEGylated *block* co-polymer (PAMAM)	90–120 nm	pH (Dox), Physical degradation (HCPT)	*In vitro* release, *in vitro* uptake, cell viability, apoptosis	57
Dox, CPT-11	PLGA, Pluronic F127, chitosan, HA w/o/w emulsion	63 nm	pH	*In vitro* release, *in vitro* uptake, cell viability, *in vivo* anti-tumor efficacy, *in vivo* biodistribution	58
Dox, 5-FU	PAMAM-polylactide Amphiphilic dendrimer	69 nm	pH	*In vitro* release, cell viability, *in vivo* anti-tumor efficacy	59
Dox, Imatinib	Folate-modified PEGylated liposomes	100–160 nm	pH	*In vitro* release, *in vitro* uptake, cell viability, *in vivo* biodistribution, *in vivo* anti-tumor efficacy	60
Dox, Erlotinib	Mesoporous silica-liposome hybrid system	100 nm	pH	*In vitro* release, *in vitro* uptake, cell viability, *in vivo* biodistribution, *in vivo* anti-tumor efficacy	61
Dox, Cur	amphiphilic poly(β-amino ester)copolymer	100 nm	pH	*In vitro* release, *in vitro* uptake, cell viability, *in vivo* biodistribution, *in vivo* anti-tumor efficacy	66
Dox, Cur	Lipid-polyacrylic acid-calcium carbonate nanoparticle	100 nm	pH	*In vitro* release, *in vitro* uptake, cell viability, *in vivo* biodistribution	67
PTX, Dox	PEG–PLGA w/o/w emulsion	240 nm	pH	*In vitro* release, cell viability	73
PTX, Dox	Folate-modified polymersomes	220 nm	pH	*In vitro* release, *in vitro* uptake, cell viability, *in vivo* biodistribution, *in vivo* anti-tumor efficacy	74
PTX, Dox	Alginate/calcium carbonate nanoparticle	150 nm	pH (Theoretical)	*In vitro* release, cell viability	75
PTX, GEM	Lipid-coated mesoporous silica nanoparticle	75 nm	pH	*In vitro* release, cell viability, *in vivo* anti-tumor efficacy	76
PTX, GEM monophosphate	cRGD-modified lipid/calcium carbonate nanoparticle	85 nm	pH (GEM monophosphate)	*In vitro* release, *in vitro* uptake, *in vivo* biodistribution, *in vivo* anti-tumor efficacy	77
PTX polymeric prodrug, GEM polymeric prodrug	Complex of cationic and anionic polymers	220 nm	pH	*In vitro* release, cell viability, *in vivo* anti-tumor efficacy	80
PTX, cisplatin	Folate-modified PEG–PLGA w/o/w emulsion	170–190 nm	pH	*In vitro* release, cell viability, *in vivo* biodistribution, *in vivo* anti-tumor efficacy	81
PTX polymeric prodrug, cisplatin	Lipid/PLGA nanoparticle	190 nm	Redox (PTX, theoretical)	*In vitro* release, *in vitro* uptake, cell viability, *in vivo* biodistribution, *in vivo* anti-tumor efficacy	82
PTX, cisplatin prodrug	TAT-modified solid lipid nanoparticle	110 nm	pH	*In vitro* release, *in vitro* uptake, cell viability, *in vivo* biodistribution, *in vivo* anti-tumor efficacy	83
CPT polymeric prodrug, GEM prodrug	Self-assembled rod-shaped nano-micelle	400–500 nm in length, 180–225 in width	Redox	*In vitro* release, *in vitro* uptake, cell viability, *in vivo* biodistribution	86
CPT-11, oxaliplatin	Liposome	200 nm	Physical release	*In vitro* release, cell viability, *in vivo* biodistribution, *in vivo* anti-tumor efficacy	87
CPT-11, oxaliplatin	o/w emulsion	130 nm	Physical release	*In vitro* release, cell viability, *in vivo* biodistribution, *in vivo* anti-tumor efficacy	88
5-FU, oxaliplatin	PLGA-based w/o/w emulsion	90 nm	Physical release	*In vitro* release, cell viability, *in vivo* anti-tumor efficacy	91
5-FU prodrug, Cur polymeric prodrug	Self-assembled polymeric nanoparticle	220 nm	pH, redox	*In vitro* release, cell viability	92

5-FU, 5-fluorouracil; CPT, camptothecin; CPT-11, irinotecan; Cur, curcumin; Dox, doxorubicin; GEM, gemcitabine; HA, hyaluronic acid; HCPT, hydroxycamptothecin; PLGA, poly(D,L-lactide-*co*-glycolide); PTX, paclitaxel.

ticle size was 85 nm. These nanoparticles were relatively small compared with paclitaxel/doxorubicin co-delivery systems.

Noh *et al.* synthesized polymeric prodrugs of paclitaxel and gemcitabine, which were poly-L-lysine–carboxylate paclitaxel and hyaluronic acid–gemcitabine, respectively.[80] These two cationic and anionic polymers formed complexes *via* electrostatic interaction, resulting in the production of particles with diameter of greater than 200 nm. He *et al.* developed an amphiphilic folic acid-modified PEG–PLGA co-polymer that encapsulated paclitaxel and cisplatin based on a double-emulsion method.[81] The particle size was 180 nm. Wang *et al.* developed lipid–polymer nanoparticles using an RGD peptide–modified s–s cleavable paclitaxel prodrug, lipids, PLGA, and cisplatin with a diameter of 190 nm.[82] Cell-penetrating peptide TAT-modified solid lipid nanoparticles containing paclitaxel and an α-tocopherol succinate-modified cisplatin prodrug developed by Liu *et al.* were about 100 nm in diameter.[83] Wang *et al.* used flavone baicalein to overcome multidrug resistance against paclitaxel and fabricated folate and hyaluronic acid dual-modified PLGA nanoparticles co-delivering paclitaxel prodrug and baicalein prodrug, with a diameter of 90 nm.[84] Gupta *et al.* prepared solid lipid nanoparticles composed of paclitaxel and erlotinib which were 200 nm in diameter.[85] Particle sizes of paclitaxel co-delivery systems ($\geq$75–200 nm) tend to be larger than those of doxorubicin co-delivery systems.

The topoisomerase I inhibitor camptothecin has been combined with not only doxorubicin but also gemcitabine for co-delivery systems. Xu *et al.* synthesized a redox-sensitive PEGylated camptothecin- and gemcitabine-polymeric prodrug forming rod-shaped nanomicelles.[86] The rod-shaped nanomicelles were approx. 400–500 nm in length and 180–225 nm in width. Zhang *et al.*'s co-delivery systems carried irinotecan and oxaliplatin.[87,88] They formulated liposomes containing irinotecan hydrochloride and oxaliplatin,[87] both of which are hydrophilic. Irinotecan was remote-loaded into oxaliplatin liposomes prepared using the ethanol injection method. The particle size was 200 nm. They also developed co-loaded o/w emulsions, in which both irinotecan and oxaliplatin were loaded inside an oil core.[88] To encapsulate hydrophilic drugs in the oil core, they used the drug–phospholipid complex technique, with resulting particle size of 130 nm. The co-loaded emulsions had superior antitumor effects compared with the mixture of single-loaded emulsions. Wang's group synthesized a PEGylated polymeric prodrug of SN-38, which is an active metabolite of irinotecan.[89] That prodrug encapsulated the hydrophobic hedgehog pathway inhibitor GDC-0449 to overcome multidrug resistance. Nanoparticle sizes were 40–80 nm. Xiao *et al.* encapsulated camptothecin and curcumin in PLGA nanoparticles with a diameter of approx. 200 nm.[90]

5-FU, a water-soluble anticancer agent, is a DNA and RNA synthesis inhibitor. Handali *et al.* formulated a 3-hydroxybutyrate-*co*-3-hydroxyvalerate/PLGA-based w/o/w double emulsion containing 5-FU and oxaliplatin with a diameter of 90 nm.[91] Sauraj *et al.* made a lipophilic 5-FU prodrug and formulated nanoparticles containing the 5-FU prodrug and a redox-sensitive curcumin prodrug which were 200 nm in diameter.[92] Feng's group prepared PEGylated liposomes containing 5-FU and the autophagy inhibitor LY294002 with diameters of 150 nm.[93]

Among other combinations, Jose *et al.* formulated liposomes containing imatinib and the selective estrogen receptor modulator tamoxifen to treat breast cancer.[94] Wang *et al.* encapsulated the multikinase inhibitor sorafenib and the flavonol antioxidant quercetin into RGD-modified lipid-PLGA nanoparticles for the treatment of hepatocellular carcinoma.[95] Zhao *et al.* formulated chitosan nanoparticles containing gefitinib and the autophagy inhibitor chloroquine.[96] Jeannot *et al.* formulated hyaluronan–poly(γ-benzyl-L-glutamate) block co-polymer micelles carrying gefitinib and the histone deacetylase inhibitor vorinostat (suberanilohydroxamic acid, SAHA).[97] Chen *et al.* prepared PEGylated polylactic acid nanoparticles containing erlotinib and fedratinib to treat erlotinib-resistant non-small cell lung cancer.[98] Nie *et al.* formulated star-shaped PLGA nanoparticles containing the proteasome inhibitor bortezomib and the microtubule stabilizer docetaxel.[99]

4. Co-delivery of Nucleic Acids or Genes with Small-Molecule Drugs

Nucleic acids and genes require delivery systems because they are too large to penetrate the plasma membrane. Small nucleic acids such as small interfering RNA (siRNA) and microRNA (miRNA) are utilized to regulate target gene expression, while genes such as plasmid DNA are utilized to express foreign genes. The molecular weight of siRNA and miRNA is 13–15 kDa and 6–7 kDa, respectively, whereas plasmid DNA molecules are huge (3000–7000 kDa) in comparison. There are other differences between small RNA and plasmid DNA, *i.e.*, the presence of a hydroxyl group and capability for chemical synthesis. In general, however, delivery systems for RNA and DNA are constructed using electrostatic interaction, which is based on their common nature (polyanion). Thus, strategies for co-delivery systems of nucleic acids and genes with small-molecule drugs are dependent on the small-molecule drugs selected. Co-delivery systems of nucleic acids and genes with small-molecule drugs are summarized in Table 2.

It was reported that free doxorubicin increased the gene expression of the commercially available liposome Lipofectamine 2000/plasmid DNA complex approx. 20-fold in A549 cells and murine lung.[100] Un *et al.* formulated a co-delivery system of doxorubicin and plasmid DNA using cationic liposomes.[101] After that, several systems delivering plasmid DNA and doxorubicin were developed, including supramolecular self-assembled oligoethylenimine-conjugated β-cyclodextrin and hyperbranched polymer (100–300 nm in size),[102] cationic pullulan-*graft*-desoxycholic acid-*graft*-polyethyleneimine (PEI) micelles (180 nm),[103] hyperbranched PEI-*graft*-polyleucine (80–120 nm),[104] redox-sensitive PEI-*graft*-poly(ε-caprolactone) (220 nm),[105] redox-sensitive polypeptide micelles (80–150 nm),[106] pH-sensitive poly-β-aminoester nanoparticles (130–190 nm),[107] and pH-sensitive mesoporous silica nanoparticles (270–330 nm).[108]

Reviewing the above, it appears difficult to prepare small nanoparticles of less than 100 nm with plasmid DNA encapsulation. On the other hand, small RNA such as siRNA and miRNA have also been co-delivered with doxorubicin, including a pH-sensitive folate-modified PEI-based doxorubicin conjugate (120 nm),[109] oligoethyleneimine-based hyperbranched polymer/hyperbranched polyglycerol (200 nm),[110] polyethylenimine-based pH-sensitive polymer (80 nm),[111] and lipid/calcium carbonate nanoparticles (100 nm).[112] Therefore,

Table 2. Co-delivery Systems Containing Nucleic Acids or Genes with Small-Molecule Drugs

Cargos (small molecule drugs)	Cargos (nucleic acids or genes)	Carriers	Particle size	Effect of cargos on gene expression	Ref.
Dox	Plasmid DNA	PEGylated liposome	105 nm	Enhanced	101
		Supramolecular self-assembled oligoethylenimine-conjugated β-Cyclodextrin and hyperbranched polymer	100–300 nm	Not tested	102
		Cationic pullulan-*graft*-desoxycholic acid-*graft*-polyethyleneimine (PEI) micelles	180 nm	Not tested	103
		Hyperbranched PEI-*graft*-polyleucine	80–120 nm	Unchanged	104
		Redox-sensitive PEI-*graft*-poly(ε-caprolactone)	220 nm	Not tested	105
		Redox-sensitive polypeptide micelles	80–150 nm	Not tested	106
		pH-sensitive poly-β-aminoester nanoparticles	130–190 nm	Not tested	107
		pH-sensitive mesoporous silica nanoparticles	270–330 nm	Not tested	108
	siRNA	pH-sensitive folate-modified PEI-based doxorubicin conjugate	120 nm	Not tested	109
		Oligoethyleneimine-based hyperbranched polymer/hyperbranched polyglycerol	200 nm	Not tested	110
		Polyethylenimine-based pH-sensitive polymer	80 nm	Not tested	111
	miRNA	Lipid/calcium carbonate nanoparticle	100 nm	Not tested	112
PTX	Plasmid DNA	Folate-modified oligoethyleneimine-γ-cyclodextrin conjugate	70–110 nm	Enhanced (KB cells), Unchanged (A549 cells)	113
		pH-sensitive hyaluronic acid-modified solid lipid nanoparticle	110–160 nm	Not tested	114
	siRNA	PDMAEMA–PCL–PDMAEMA polymer	50–130 nm	Improved knockdown	115
		PEGylated polyaspartic acid-modified PEI-polylactic acid nanoparticle	60–100 nm	Improved knockdown	116
CPT	Plasmid DNA	Redox- and pH-sensitive polymer	115–180 nm	Enhanced	117
5-FU	siRNA	Layered aluminum/magnesium hydroxide nanoparticle	85 nm	Improved knockdown	119
Docetaxel	Plasmid DNA	Redox-sensitive polymeric micelle	100–250 nm	Slightly decreased	120
Erlotinib	Plasmid DNA	Polyamidoamine dendrimers	340–500 nm	Slightly decreased	121
Methotrexate	Plasmid DNA	Hyperbranched poly(amido amine) polymer	200 nm	Slightly decreased	122
	Plasmid DNA	Transferrin-modified hyperbranched poly(amido amine) polymer	180–240 nm	Slightly decreased	123

5-FU, 5-fluorouracil; CPT, camptothecin; Dox, doxorubicin; PTX, paclitaxel.

small RNA-containing nanoparticles tend to have smaller particle size than plasmid DNA-containing ones. On the contrary, the effect of doxorubicin on RNA interference is still unclear since most researchers did not examine it. The incorporation of doxorubicin might have off-target effects, *i.e.*, nonspecific decreases in target protein expression.

Paclitaxel is also co-delivered with plasmid DNA and RNA. Zhao *et al.* demonstrated that co-delivery of paclitaxel with plasmid DNA *via* an oligoethyleneimine-γ-cyclodextrin conjugate enhanced gene expression, especially with folate ligand modification, in KB cells, whereas it did not enhance gene expression in A549 cells.[113] The particle sizes of the complexes were 70–110 nm. Yu *et al.* developed pH-sensitive hyaluronic acid-modified solid lipid nanoparticles carrying plasmid DNA and paclitaxel (110–160 nm in diameter).[114] For RNA delivery, Zhu *et al.* synthesized a PDMAEMA–PCL–PDMAEMA polymer containing vascular endothelial growth factor (VEGF) siRNA and paclitaxel, and the co-delivery improved VEGF knockdown efficiency (particle size, 50–130 nm).[115] Jin's group developed PEGylated polyaspartic acid-modified PEI–polylactic acid nanoparticles carrying survivin siRNA and paclitaxel (60–100 nm).[116] The co-delivery of survivin siRNA and paclitaxel exhibited synergistic effects on survivin knockdown.

Among other combinations, camptothecin was co-delivered with plasmid DNA[117] and siRNA.[118] According to the reports, camptothecin seemed to enhance the transfection efficiency of both plasmid DNA and siRNA. Li *et al.* co-delivered 5-FU and Bcl-2 siRNA using layered aluminum/magnesium hydroxide nanoparticles in MCF-7 cells, which suppressed Bcl-2 protein expression more strongly than nanoparticles containing Bcl-2 alone.[119] On the other hand, co-delivery of plasmid DNA with docetaxel using redox-sensitive polymeric micelles slightly decreased the percentage of transfection in MCF-7 cells, while the combination inhibited *in vivo* tumor growth.[120] Co-delivery of plasmid DNA with erlotinib using polyamidoamine dendrimers also slightly decreased the percentage of transfection of PC9 and H1975 cells.[121] Similar results were obtained with the plasmid DNA and methotrexate combination using hyperbranched poly(amido amine) polymer.[122,123] Thus, whether a synergistic effect is obtained in terms of transfection efficiency depends on the partner chemotherapeutic drugs.

There were various other attempts to improve transfection efficiency using small-molecule drugs. It is known that the steroidal antiinflammatory drug dexamethasone not only suppresses inflammation by transfection but also enhances gene expression.[124] Co-delivery of dexamethasone also increased the transfection efficiency of lipopolyplexes using cationic liposomes and branched PEI.[125] Dexamethasone–palmitate also enhanced transfection efficiency of lipid nanoparticles carrying mRNA.[126] On the other hand, antioxidants also enhance transfection efficiency. The antioxidant trolox enhanced the transfection efficiency of lipoplexes *in vitro*.[127] Togashi *et al.* demonstrated that a vitamin E scaffold in ssPalm, which is a redox-sensitive ionizable lipid, had superior transfection efficiency of plasmid DNA compared with the myristoyl version of ssPalm.[128] The *in vivo* transfection efficiency was further enhanced by co-delivery of dexamethasone–palmitate. We also demonstrated that the antioxidant edaravone enhanced lipofection in HepG2 cells.[129] The cell toxicity of lipoplexes was reduced by edaravone. For *in vivo* application, we developed edaravone-loaded liposomes, decreasing the effective dose of edaravone.

5. Co-delivery of Proteins with Small-Molecule Drugs

The delivery of proteins is more complicated than that of nucleic acids since the nature of proteins is highly dependent on each protein type. There are several examples of co-delivery systems for proteins. Wu *et al.* synthesized a folic acid-modified chitosan-based polymeric prodrug of doxorubicin complexed with recombinant interleukin-2 (IL-2).[130] The particle size was 110–200 nm. The *in vitro* efficacy of IL-2 co-delivery with doxorubicin was limited, while IL-2 co-delivery improved *in vivo* antitumor effects. Yin *et al.* developed a co-delivery system of a pH-sensitive doxorubicin polymeric prodrug with interferon-γ using thermosensitive nanoparticles (120–150 nm).[131]

However, there seems to be no reasonable theory for the co-delivery of cytokines with small-molecule anticancer drugs by incorporation into single nanoparticles. Co-delivery of the anti-epidermal growth factor receptor antibody cetuximab with paclitaxel using carbon-based nanodiamonds increased paclitaxel uptake.[132] Such a combination, *i.e.*, delivery systems of small-molecule drugs using antibodies, are similar to antibody–drug conjugates.

On the other hand, Kim *et al.* demonstrated synergistic cytotoxic effects of the co-delivery of paclitaxel and caspase-3 protein using gold nanoparticle-stabilized nanocapsules (130–140 nm).[133] Similar combinations, *i.e.*, chitosan-based paclitaxel nanocapsules complexed with caspase-3 (190–220 nm) were also reported.[134] The incorporation of paclitaxel and caspase-3 protein into a single nanoparticle seems to be reasonable since caspase-3 acts inside target cells. We developed a co-delivery system of superoxide dismutase with paclitaxel using RGD-modified lipid/calcium carbonate nanoparticles (130 nm).[135] The combination of superoxide dismutase with paclitaxel had synergistic effects. Synchronized biodistribution of superoxide dismutase and paclitaxel by RGD-modified nanoparticles exhibited superior *in vivo* antitumor effects compared with the free combination and unmodified nanoparticles.

6. Preparation Methods for Co-delivery Systems

Preparation methods for nanoparticles delivering multiple drugs are slightly complicated compared with single-drug formulations. Encapsulating drugs with different properties generally requires more than two steps (for example, the double-emulsion method, remote-loading method, and complex formation). Formation of self-assembled nanocarriers using a polymeric prodrug may require only one step, although the synthesis of polymeric prodrugs is cumbersome. However, the ethanol (alcohol) injection method makes preparation simple, *i.e.*, with real one-step formation such as lipid nanoparticles. We applied the ethanol injection method to the formation of polymer-assisted lipid-coated calcium carbonate nanoparticles.[67,135] This preparation method is simpler than that for the similar nanoparticle LCP, where hydration of a lipid film with a preformed calcium phosphate core is necessary.[78,79]

It is generally difficult to prepare small nanoparticles of less than 100 nm in diameter. Conventional liposome preparation methods such as lipid film hydration and ethanol injection with standard mixing methods (stirring, pipetting, *etc.*) produce relatively large nanoparticles of around 100 nm or greater in diameter. Microfluidics is therefore a useful mixing method. Using microfluidics with ethanol injection allows the production of small lipid nanoparticles of less than 50 nm in diameter such as limited-sized liposomes.[136,137] Ethanol injection and microfluidic-based mixing combinations can theoretically be applied to our lipid/calcium carbonate nanoparticles since their formation is based on one-step mixing of alcohol and water phases.

Table 3. Advantages and Disadvantages of Co-delivery Systems

Merits	Demerits
Synchronized biodistribution	Difficulty in synchronized drug release
Synergistic effects	Difficulty in reducing particle size
Reducing side effects	Complicated preparation
Simplifying clinical procedures	Difficulty in changing drug ratio in clinic
Improving quality of life	Difficulty in reproducing complicated regimens

7. Prospects for Co-delivery

Table 3 summarizes the advantages and disadvantages of co-delivery systems. The main advantage is potential synergistic effects based on synchronized biodistribution and depending on the drug combination. Synergistic effects are also expected in the case of free-combination and multiple-delivery system combination. However, the disposition of each drug/delivery system may differ. Theoretically, spatiotemporal biodistribution can be synchronized in co-delivery systems, although maintaining synchronized biodistribution of drugs with different properties is very difficult. Moreover, it is also difficult to reproduce complicated regimens such as FOLFIRI, where 5-FU is continuously infused for 2 d after 2-h infusion of levofolinate and irinotecan. In contrast, co-delivery systems can simplify treatment regimens, which is a major advantage for improving quality of life and simplifying clinical procedures.

A particle size of less than 200 nm is sufficiently small for use in highly permeable murine tumor models,[138,139] but Cabral *et al.* reported that only 30-nm polymeric micelles penetrated poorly permeable pancreatic cancer, whereas polymeric micelles greater than 50 nm in diameter could not.[140] The EPR effect works in rodents but not in humans based on clinical evidence.[141] Therefore, the target particle size has decreased from 200 nm to 50 nm over the past decade. Because the amount of cargo in a single 50-nm particle is 64-fold less than that in a 200-nm one, it is necessary to deliver 64-fold more of the smaller particles to achieve the same drug dose. This is known as the "particle size dilemma."

Immune cells can migrate into tumor tissues, and immunotherapy is therefore a reasonable strategy to attack tumors. In the near future, researchers will investigate the co-delivery of immune checkpoint inhibitors with immunostimulatory drugs. For anti-PD-1 antibodies, T cells are the target. However, there are few or no immunostimulatory drugs targeting T cells. Taking cell-to-cell communication into account, immunologic adjuvants such as Toll-like receptor agonists may be a useful strategy. In contrast, the target of anti-PD-L1 antibodies is PD-L1-positive cancer cells, and thus chemotherapy delivered to those cells may lead to target cell-selective immunogenic cell death. Such strategies have already been tested using an antibody-drug conjugate with doxorubicin,[142] and co-delivery systems of anti-PD-L1 antibodies with chemotherapeutic agents will likely become available.

Recently, it has been revealed that cancer cell-derived ex-

tracellular vesicles (including exosomes) play a pivotal role in cancer immunity. Extracellular vesicles are expected to be useful DDS carriers,[143] since cancer cell-derived ones are basically immunosuppressive.[144] However, LATS1/2 double-deficient cancer cells release immune-stimulating extracellular vesicles.[145,146] The targets of immune-stimulating extracellular vesicles are immune cells such as dendritic cells, and therefore immunologic adjuvants are candidate cargos. Co-delivery systems of immunologic adjuvants with extracellular vesicles from LATS1/2 double-deficient cancer cells are promising. Immune cells such as macrophages, dendritic cells, and NK cells produce the exosomes attacking cancer cells.[147] Sato *et al.* investigated hybrid exosomes with liposomes.[148] Using such techniques, co-delivery of drugs using exosomes can be achieved.

Cells have also been used as DDS carriers.[149] It was reported that *ex vivo* addition of a transforming growth factor β (TGF-β) receptor kinase inhibitor to NK cells resulted in drastic improvement of anticancer efficacy.[150] Overall, various co-delivery systems can be expected in the future.

Not only the development but also the appropriate evaluation of DDS is important.[151] Analyses of biodistribution,[67,135] local disposition,[152–155] interactions with blood components,[156,157] and intracellular fate[158] will help validate the usefulness of co-delivery systems and then improve them. Confirmation of the synergistic effects of co-delivered drugs is a particularly important issue. Appropriate evaluation of synergistic effects using a combination index[159] is needed to conclude whether the effects are synergistic or additive.

8. Conclusion

Co-delivery systems of multiple drugs are a promising approach to treat cancer. Synergistic actions with decreased side effects are a main goal of co-delivery systems. Various combinations of drugs with different mechanisms of action are available, although the combinations do not always match clinical regimens. Co-delivery of drugs with different properties is relatively difficult, but some researchers have been able to create such co-delivery systems. New modalities such as nucleic acids, exosomes, and cells are also essentially compatible with co-delivery. We hope that advances in DDS will overcome cancer in the near future.

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Conflict of Interest The authors declare no conflict of interest.

References

1) Dagenais G. R., Leong D. P., Rangarajan S., *et al.*, *Lancet*, **395**, 785–794 (2020).
2) Allemani C., Matsuda T., Di Carlo V., *et al.*, *Lancet*, **391**, 1023–1075 (2018).
3) Morrison W. B., *J. Vet. Intern. Med.*, **24**, 1249–1262 (2010).
4) Nygren P., *Acta Oncol.*, **40**, 166–174 (2001).
5) Yazdi M. H., Faramarzi M. A., Nikfar S., Abdollahi M., *Biomed. Pharmacother.*, **95**, 1556–1564 (2017).
6) Huang M., Shen A., Ding J., Geng M., *Trends Pharmacol. Sci.*, **35**, 41–50 (2014).
7) Buss N. A., Henderson S. J., McFarlane M., Shenton J. M., de Haan L., *Curr. Opin. Pharmacol.*, **12**, 615–622 (2012).
8) Elgundi Z., Reslan M., Cruz E., Sifniotis V., Kayser V., *Adv. Drug Deliv. Rev.*, **122**, 2–19 (2017).
9) Lee A., Sun S., Sandler A., Hoang T., *Curr. Opin. Chem. Biol.*, **44**, 56–65 (2018).
10) Holstein S. A., Lunning M. A., *Clin. Pharmacol. Ther.*, **107**, 112–122 (2020).
11) Ma S., Li X., Wang X., Cheng L., Li Z., Zhang C., Ye Z., Qian Q., *Int. J. Biol. Sci.*, **15**, 2548–2560 (2019).
12) Bayat Mokhtari R., Homayouni T. S., Baluch N., Morgatskaya E., Kumar S., Das B., Yeger H., *Oncotarget*, **8**, 38022–38043 (2017).
13) Qin S. Y., Cheng Y. J., Lei Q., Zhang A. Q., Zhang X. Z., *Biomaterials*, **171**, 178–197 (2018).
14) Yang F., Jin C., Fu D. L., Warshaw A. L., *World J. Gastroenterol.*, **25**, 2839–2845 (2019).
15) Von Hoff D. D., Ervin T., Arena F. P., *et al.*, *N. Engl. J. Med.*, **369**, 1691–1703 (2013).
16) Klaiber U., Leonhardt C. S., Strobel O., Tjaden C., Hackert T., Neoptolemos J. P., *Langenbecks Arch. Surg.*, **403**, 917–932 (2018).
17) Kyi C., Postow M. A., *Immunotherapy*, **8**, 821–837 (2016).
18) Wang L., Yao R., Zhang L., Fan C., Ma L., Liu J., *Int. Immunopharmacol.*, **70**, 498–503 (2019).
19) Xu J., Wang Y., Shi J., Liu J., Li Q., Chen L., *Oncol. Lett.*, **16**, 2063–2070 (2018).
20) Wu D., Si M., Xue H. Y., Wong H. L., *Int. J. Nanomedicine*, **12**, 5879–5892 (2017).
21) Abu Lila A. S., Ishida T., *Biol. Pharm. Bull.*, **40**, 1–10 (2017).
22) Oku N., *Biol. Pharm. Bull.*, **40**, 119–127 (2017).
23) Geszke-Moritz M., Moritz M., *Mater. Sci. Eng. C*, **68**, 982–994 (2016).
24) Nishiyama N., Matsumura Y., Kataoka K., *Cancer Sci.*, **107**, 867–874 (2016).
25) Sharma A., Garg T., Aman A., Panchal K., Sharma R., Kumar S., Markandeywar T., *Artif. Cells Nanomed. Biotechnol.*, **44**, 165–177 (2016).
26) Mir M., Ahmed N., Rehman A. U., *Colloids Surf. B Biointerfaces*, **159**, 217–231 (2017).
27) Kawakami S., Hashida M., *J. Control. Release*, **190**, 542–555 (2014).
28) Hagimori M., Fuchigami Y., Kawakami S., *Chem. Pharm. Bull.*, **65**, 618–624 (2017).
29) Liu D., Yang F., Xiong F., Gu N., *Theranostics*, **6**, 1306–1323 (2016).
30) Fumoto S., Kawakami S., *Biol. Pharm. Bull.*, **37**, 212–216 (2014).
31) Qi S. S., Sun J. H., Yu H. H., Yu S. Q., *Drug Deliv.*, **24**, 1909–1926 (2017).
32) Teo P. Y., Cheng W., Hedrick J. L., Yang Y. Y., *Adv. Drug Deliv. Rev.*, **98**, 41–63 (2016).
33) Zununi Vahed S., Salehi R., Davaran S., Sharifi S., *Mater. Sci. Eng. C*, **71**, 1327–1341 (2017).
34) Li M., Du C., Guo N., Teng Y., Meng X., Sun H., Li S., Yu P., Galons H., *Eur. J. Med. Chem.*, **164**, 640–653 (2019).
35) Felgner P. L., Gadek T. R., Holm M., Roman R., Chan H. W., Wenz M., Northrop J. P., Ringold G. M., Danielsen M., *Proc. Natl. Acad. Sci. U.S.A.*, **84**, 7413–7417 (1987).
36) Hattori Y., Hara E., Shingu Y., Minamiguchi D., Nakamura A., Arai S., Ohno H., Kawano K., Fujii N., Yonemochi E., *Biol. Pharm. Bull.*, **38**, 30–38 (2015).
37) Li G., Liu J., Pang Y., Wang R., Mao L., Yan D., Zhu X., Sun J., *Biomacromolecules*, **12**, 2016–2026 (2011).
38) Oishi M., Nagasaki Y., Itaka K., Nishiyama N., Kataoka K., *J. Am. Chem. Soc.*, **127**, 1624–1625 (2005).
39) Miyata K., Oba M., Nakanishi M., Fukushima S., Yamasaki Y., Koyama H., Nishiyama N., Kataoka K., *J. Am. Chem. Soc.*, **130**, 16287–16294 (2008).
40) Shi L., Ding K., Sun X., Zhang L., Zeng T., Yin Y., Zheng H., *J. Biomater. Sci. Polym. Ed.*, **27**, 472–489 (2016).

41) Lee Y., Thompson D. H., *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.*, **9**, e1450 (2017).
42) Oerlemans C., Bult W., Bos M., Storm G., Nijsen J. F., Hennink W. E., *Pharm. Res.*, **27**, 2569–2589 (2010).
43) Bai S., Hou M., Shi X., Chen J., Ma X., Gao Y. E., Wang Y., Xue P., Kang Y., Xu Z., *Carbohydr. Polym.*, **193**, 153–162 (2018).
44) Webber M. J., Langer R., *Chem. Soc. Rev.*, **46**, 6600–6620 (2017).
45) Li J., Loh X. J., *Adv. Drug Deliv. Rev.*, **60**, 1000–1017 (2008).
46) Pedrosa S. S., Gonçalves C., David L., Gama M., *Macromol. Biosci.*, **14**, 1556–1568 (2014).
47) Sharma A., Garg T., Aman A., Panchal K., Sharma R., Kumar S., Markandeywar T., *Artif. Cells Nanomed. Biotechnol.*, **44**, 165–177 (2016).
48) Ding F., Mou Q., Ma Y., Pan G., Guo Y., Tong G., Choi C. H. J., Zhu X., Zhang C., *Angew. Chem. Int. Ed.*, **57**, 3064–3068 (2018).
49) Huang D., Qian H., Qiao H., Chen W., Feijen J., Zhong Z., *Expert Opin. Drug Deliv.*, **15**, 703–716 (2018).
50) Bender U., Rho Y. S., Barrera I., Aghajanyan S., Acoba J., Kavan P., *Curr. Oncol.*, **26** (Suppl. 1), S43–S52 (2019).
51) Neugut A. I., Lin A., Raab G. T., Hillyer G. C., Keller D., O'Neil D. S., Accordino M. K., Kiran R. P., Wright J., Hershman D. L., *Clin. Colorectal Cancer*, **18**, 133–140 (2019).
52) Müller I., Niethammer D., Bruchelt G., *Int. J. Mol. Med.*, **1**, 491–494 (1998).
53) Wang Q., Ren T., Zhao J., Wong C. H., Chan H. Y. E., Zuo Z., *J. Pharm. Biomed. Anal.*, **178**, 112946 (2020).
54) Tai W., Mo R., Lu Y., Jiang T., Gu Z., *Biomaterials*, **35**, 7194–7203 (2014).
55) Maeda H., Wu J., Sawa T., Matsumura Y., Hori K., *J. Control. Release*, **65**, 271–284 (2000).
56) Gao Y. E., Bai S., Ma X., Zhang X., Hou M., Shi X., Huang X., Chen J., Wen F., Xue P., Kang Y., Xu Z., *Colloids Surf. B Biointerfaces*, **183**, 110428 (2019).
57) Zhang Y., Xiao C., Li M., Chen J., Ding J., He C., Zhuang X., Chen X., *Macromol. Biosci.*, **13**, 584–594 (2013).
58) Wang H., Agarwal P., Zhao S., Xu R. X., Yu J., Lu X., He X., *Biomaterials*, **72**, 74–89 (2015).
59) Han R., Sun Y., Kang C., Sun H., Wei W., *J. Drug Target.*, **25**, 140–148 (2017).
60) Chen Y., Cheng Y., Zhao P., Zhang S., Li M., He C., Zhang X., Yang T., Yan R., Ye P., Ma X., Xiang G., *Int. J. Pharm.*, **542**, 266–279 (2018).
61) He Y., Su Z., Xue L., Xu H., Zhang C., *J. Control. Release*, **229**, 80–92 (2016).
62) Lakkadwala S., Dos Santos Rodrigues B., Sun C., Singh J., *J. Control. Release*, **307**, 247–260 (2019).
63) Goel A., Aggarwal B. B., *Nutr. Cancer*, **62**, 919–930 (2010).
64) Mohajeri M., Sahebkar A., *Crit. Rev. Oncol. Hematol.*, **122**, 30–51 (2018).
65) Jannin V., Chevrier S., Michenaud M., Dumont C., Belotti S., Chavant Y., Demarne F., *Int. J. Pharm.*, **495**, 385–392 (2015).
66) Zhang J., Li J., Shi Z., Yang Y., Xie X., Lee S. M., Wang Y., Leong K. W., Chen M., *Acta Biomater.*, **58**, 349–364 (2017).
67) Peng J., Fumoto S., Miyamoto H., Chen Y., Kuroda N., Nishida K., *J. Drug Target.*, **25**, 704–714 (2017).
68) Chen C., Tao R., Ding D., Kong D., Fan A., Wang Z., Zhao Y., *Eur. J. Pharm. Sci.*, **107**, 16–23 (2017).
69) Tao X., Gou J., Zhang Q., Tan X., Ren T., Yao Q., Tian B., Kou L., Zhang L., Tang X., *Biomater. Sci.*, **6**, 1869–1881 (2018).
70) Skrott Z., Mistrik M., Andersen K. K., *et al.*, *Nature* (London), **552**, 194–199 (2017).
71) Skrott Z., Majera D., Gursky J., Buchtova T., Hajduch M., Mistrik M., Bartek J., *Oncogene*, **38**, 6711–6722 (2019).
72) Zhu Z., *Mol. Pharm.*, **11**, 776–786 (2014).
73) Wang H., Zhao Y., Wu Y., Hu Y. L., Nan K., Nie G., Chen H., *Biomaterials*, **32**, 8281–8290 (2011).
74) Zhu D., Wu S., Hu C., Chen Z., Wang H., Fan F., Qin Y., Wang C., Sun H., Leng X., Kong D., Zhang L., *Acta Biomater.*, **58**, 399–412 (2017).
75) Wu J. L., Wang C. Q., Zhuo R. X., Cheng S. X., *Colloids Surf. B Biointerfaces*, **123**, 498–505 (2014).
76) Meng H., Wang M., Liu H., Liu X., Situ A., Wu B., Ji Z., Chang C. H., Nel A. E., *ACS Nano*, **9**, 3540–3557 (2015).
77) Zhang J., Zhang P., Zou Q., Li X., Fu J., Luo Y., Liang X., Jin Y., *Molecules*, **23**, E2906 (2018).
78) Li J., Chen Y. C., Tseng Y. C., Mozumdar S., Huang L., *J. Control. Release*, **142**, 416–421 (2010).
79) Zhang Y., Kim W. Y., Huang L., *Biomaterials*, **34**, 3447–3458 (2013).
80) Noh I., Kim H. O., Choi J., Choi Y., Lee D. K., Huh Y. M., Haam S., *Biomaterials*, **53**, 763–774 (2015).
81) He Z., Huang J., Xu Y., Zhang X., Teng Y., Huang C., Wu Y., Zhang X., Zhang H., Sun W., *Oncotarget*, **6**, 42150–42168 (2015).
82) Wang G., Wang Z., Li C., Duan G., Wang K., Li Q., Tao T., *Biomed. Pharmacother.*, **106**, 275–284 (2018).
83) Liu B., Han L., Liu J., Han S., Chen Z., Jiang L., *Int. J. Nanomedicine*, **12**, 955–968 (2017).
84) Wang W., Xi M., Duan X., Wang Y., Kong F., *Int. J. Nanomedicine*, **10**, 3737–3750 (2015).
85) Gupta B., Poudel B. K., Regmi S., Pathak S., Ruttala H. B., Gautam M., An G. J., Jeong J. H., Choi H. G., Yong C. S., Kim J. O., *Pharm. Res.*, **35**, 96 (2018).
86) Xu Y., Huang Y., Lu W., Liu S., Xiao Y., Yu J., *Eur. J. Pharm. Biopharm.*, **144**, 193–206 (2019).
87) Zhang B., Wang T., Yang S., Xiao Y., Song Y., Zhang N., Garg S., *J. Control. Release*, **238**, 10–21 (2016).
88) Zhang B., Song Y., Wang T., Yang S., Zhang J., Liu Y., Zhang N., Garg S., *Int. J. Nanomedicine*, **12**, 2871–2886 (2017).
89) Wang L., Liu X., Zhou Q., Sui M., Lu Z., Zhou Z., Tang J., Miao Y., Zheng M., Wang W., Shen Y., *Biomaterials*, **144**, 105–118 (2017).
90) Xiao B., Si X., Han M. K., Viennois E., Zhang M., Merlin D., *J. Mater. Chem. B*, **3**, 7724–7733 (2015).
91) Handali S., Moghimipour E., Rezaei M., Saremy S., Dorkoosh F. A., *Int. J. Biol. Macromol.*, **124**, 1299–1311 (2019).
92) Sauraj V. K., Kumar B., Priyadarshi R., Deeba F., Kulshreshtha A., Kumar A., Agrawal G., Gopinath P., Negi Y. S., *Mater. Sci. Eng. C*, **107**, 110356 (2020).
93) Feng Y., Gao Y., Wang D., Xu Z., Sun W., Ren P., *Nanoscale Res. Lett.*, **13**, 325 (2018).
94) Jose A., Ninave K. M., Karnam S., Venuganti V. V. K., *J. Liposome Res.*, **29**, 153–162 (2019).
95) Wang C., Su L., Wu C., Wu J., Zhu C., Yuan G., *Drug Dev. Ind. Pharm.*, **42**, 1938–1944 (2016).
96) Zhao L., Yang G., Shi Y., Su C., Chang J., *J. Nanobiotechnology*, **13**, 57 (2015).
97) Jeannot V., Gauche C., Mazzaferro S., Couvet M., Vanwonterghem L., Henry M., Didier C., Vollaire J., Josserand V., Coll J. L., Schatz C., Lecommandoux S., Hurbin A., *J. Control. Release*, **275**, 117–128 (2018).
98) Chen D., Zhang F., Wang J., He H., Duan S., Zhu R., Chen C., Yin L., Chen Y., *Front. Pharmacol.*, **9**, 1214 (2018).
99) Nie J., Cheng W., Peng Y., Liu G., Chen Y., Wang X., Liang C., Tao W., Wei Y., Zeng X., Mei L., *Drug Deliv.*, **24**, 1124–1138 (2017).
100) Griesenbach U., Meng C., Farley R., Gardner A., Brake M. A., Frankel G. M., Gruenert D. C., Cheng S. H., Scheule R. K., Alton E. W., *Biomaterials*, **30**, 1971–1977 (2009).
101) Un K., Kono Y., Yoshida M., Yamashita F., Kawakami S., Hashida M., *Pharmazie*, **67**, 400–405 (2012).
102) Zhou X., Xu L., Xu J., Wu J., Kirk T. B., Ma D., Xue W., *ACS Appl. Mater. Interfaces*, **10**, 35812–35829 (2018).

103) Chen L., Ji F., Bao Y., Xia J., Guo L., Wang J., Li Y., *Mater. Sci. Eng. C*, **70**, 418–429 (2017).
104) Li Y., Zhang X., Zhang J., Mu X., Duan Q., Wang T., Tian H., *J. Mater. Sci. Mater. Med.*, **29**, 47 (2018).
105) Davoodi P., Srinivasan M. P., Wang C. H., *Acta Biomater.*, **39**, 79–93 (2016).
106) Hu C., Gu F., Tai Z., Yao C., Gong C., Xia Q., Gao Y., Gao S., *Oncotarget*, **7**, 61832–61844 (2016).
107) Tang S., Yin Q., Zhang Z., Gu W., Chen L., Yu H., Huang Y., Chen X., Xu M., Li Y., *Biomaterials*, **35**, 6047–6059 (2014).
108) Li Z., Zhang L., Tang C., Yin C., *Pharm. Res.*, **34**, 2829–2841 (2017).
109) Dong D. W., Xiang B., Gao W., Yang Z. Z., Li J. Q., Qi X. R., *Biomaterials*, **34**, 4849–4859 (2013).
110) Jia H. Z., Zhang W., Zhu J. Y., Yang B., Chen S., Chen G., Zhao Y. F., Feng J., Zhang X. Z., *J. Control. Release*, **216**, 9–17 (2015).
111) Xu C., Wang P., Zhang J., Tian H., Park K., Chen X., *Small*, **11**, 4321–4333 (2015).
112) Zhao P., Wu S., Cheng Y., You J., Chen Y., Li M., He C., Zhang X., Yang T., Lu Y., Lee R. J., He X., Xiang G., *Nanomedicine*, **13**, 2507–2516 (2017).
113) Zhao F., Yin H., Li J., *Biomaterials*, **35**, 1050–1062 (2014).
114) Yu D., Li W., Zhang Y., Zhang B., *Biomed. Pharmacother.*, **83**, 1428–1435 (2016).
115) Zhu C., Jung S., Luo S., Meng F., Zhu X., Park T. G., Zhong Z., *Biomaterials*, **31**, 2408–2416 (2010).
116) Jin M., Jin G., Kang L., Chen L., Gao Z., Huang W., *Int. J. Nanomedicine*, **13**, 2405–2426 (2018).
117) Chen M., Zhang Y., Chen Z., Xie S., Luo X., Li X., *Acta Biomater.*, **49**, 444–455 (2017).
118) Samson A. A. S., Park S., Kim S. Y., Min D. H., Jeon N. L., Song J. M., *J. Liposome Res.*, **29**, 44–52 (2019).
119) Li L., Gu W., Chen J., Chen W., Xu Z. P., *Biomaterials*, **35**, 3331–3339 (2014).
120) Kang Y., Lu L., Lan J., Ding Y., Yang J., Zhang Y., Zhao Y., Zhang T., Ho R. J. Y., *Acta Biomater.*, **68**, 137–153 (2018).
121) Lv T., Li Z., Xu L., Zhang Y., Chen H., Gao Y., *Acta Biomater.*, **76**, 257–274 (2018).
122) Tang Q., Ma X., Zhang Y., Cai X., Xue W., Ma D., *Acta Biomater.*, **69**, 277–289 (2018).
123) Liu T., Wu X., Chen S., Wu P., Han H., Zhang H., Li J., Li G., Zhang S., *Drug Deliv.*, **26**, 1280–1291 (2019).
124) Tan Y., Li S., Pitt B. R., Huang L., *Hum. Gene Ther.*, **10**, 2153–2161 (1999).
125) Malaekeh-Nikouei B., Gholami L., Asghari F., Askarian S., Barzegar S., Rezaee M., Kazemi Oskuee R., *Colloids Surf. B Biointerfaces*, **165**, 252–261 (2018).
126) Ohto T., Konishi M., Tanaka H., Onomoto K., Yoneyama M., Nakai Y., Tange K., Yoshioka H., Akita H., *Biol. Pharm. Bull.*, **42**, 299–302 (2019).
127) Luo X., Belcastro R., Cabacungan J., Hannam V., Negus A., Wen Y., Plumb J., Hu J., Steer B., Koehler D. R., Downey G. P., Tanswell A. K., *Am. J. Physiol. Lung Cell. Mol. Physiol.*, **284**, L817–L825 (2003).
128) Togashi R., Tanaka H., Nakamura S., Yokota H., Tange K., Nakai Y., Yoshioka H., Harashima H., Akita H., *J. Control. Release*, **279**, 262–270 (2018).
129) Wang S., Fumoto S., Miyamoto H., Tanaka M., Nishida K., *Int. J. Pharm.*, **548**, 173–181 (2018).
130) Wu J., Tang C., Yin C., *Acta Biomater.*, **47**, 81–90 (2017).
131) Yin Y., Hu Q., Xu C., Qiao Q., Qin X., Song Q., Peng Y., Zhao Y., Zhang Z., *Mol. Pharm.*, **15**, 4161–4172 (2018).
132) Lin Y. W., Raj E. N., Liao W. S., Lin J., Liu K. K., Chen T. H., Cheng H. C., Wang C. C., Li L. Y., Chen C., Chao J. I., *Sci. Rep.*, **7**, 9814 (2017).
133) Kim C. S., Mout R., Zhao Y., Yeh Y. C., Tang R., Jeong Y., Duncan B., Hardy J. A., Rotello V. M., *Bioconjug. Chem.*, **26**, 950–954 (2015).
134) Wu D. Y., Ma Y., Hou X. S., Zhang W. J., Wang P., Chen H., Li B., Zhang C., Ding Y., *Carbohydr. Polym.*, **157**, 1470–1478 (2017).
135) Peng J. Q., Fumoto S., Suga T., Miyamoto H., Kuroda N., Kawakami S., Nishida K., *J. Control. Release*, **302**, 42–53 (2019).
136) Zhigaltsev I. V., Belliveau N., Hafez I., Leung A. K., Huft J., Hansen C., Cullis P. R., *Langmuir*, **28**, 3633–3640 (2012).
137) Carugo D., Bottaro E., Owen J., Stride E., Nastruzzi C., *Sci. Rep.*, **6**, 25876 (2016).
138) Maruyama K., *Adv. Drug Deliv. Rev.*, **63**, 161–169 (2011).
139) Bertrand N., Wu J., Xu X., Kamaly N., Farokhzad O. C., *Adv. Drug Deliv. Rev.*, **66**, 2–25 (2014).
140) Cabral H., Matsumoto Y., Mizuno K., Chen Q., Murakami M., Kimura M., Terada Y., Kano M. R., Miyazono K., Uesaka M., Nishiyama N., Kataoka K., *Nat. Nanotechnol.*, **6**, 815–823 (2011).
141) Danhier F., *J. Control. Release*, **244** (Pt A), 108–121 (2016).
142) Sau S., Petrovici A., Alsaab H. O., Bhise K., Iyer A. K., *Cancers*, **11**, E232 (2019).
143) Tominaga N., Yoshioka Y., Ochiya T., *Adv. Drug Deliv. Rev.*, **95**, 50–55 (2015).
144) Czernek L., Düchler M., *Arch. Immunol. Ther. Exp.*, **65**, 311–323 (2017).
145) Moroishi T., Hayashi T., Pan W. W., Fujita Y., Holt M. V., Qin J., Carson D. A., Guan K. L., *Cell*, **167**, 1525–1539.e17 (2016).
146) Yamauchi T., Moroishi T., *Cells*, **8**, E398 (2019).
147) Shen M., Ren X., *Cancer Lett.*, **431**, 115–122 (2018).
148) Sato Y. T., Umezaki K., Sawada S., Mukai S. A., Sasaki Y., Harada N., Shiku H., Akiyoshi K., *Sci. Rep.*, **6**, 21933 (2016).
149) Cheng S., Nethi S. K., Rathi S., Layek B., Prabha S., *J. Pharmacol. Exp. Ther.*, **370**, 231–241 (2019).
150) Otegbeye F., Ojo E., Moreton S., Mackowski N., Lee D. A., de Lima M., Wald D. N., *PLOS ONE*, **13**, e0191358 (2018).
151) Fumoto S., Nishida K., *Chem. Pharm. Bull.*, **65**, 642–648 (2017).
152) Fumoto S., Nakadori F., Kawakami S., Nishikawa M., Yamashita F., Hashida M., *Pharm. Res.*, **20**, 1452–1459 (2003).
153) Fumoto S., Kawakami S., Ishizuka M., Nishikawa M., Yamashita F., Hashida M., *Drug Metab. Pharmacokinet.*, **18**, 230–237 (2003).
154) Fumoto S., Kawakami S., Ito Y., Shigeta K., Yamashita F., Hashida M., *Mol. Ther.*, **10**, 719–729 (2004).
155) Fumoto S., Nishimura K., Nishida K., Kawakami S., *PLOS ONE*, **11**, e0148233 (2016).
156) Fumoto S., Kawakami S., Shigeta K., Higuchi Y., Yamashita F., Hashida M., *J. Pharmacol. Exp. Ther.*, **315**, 484–493 (2005).
157) Yoshikawa N., Fumoto S., Nakashima M., Shimokawa K., Miyamoto H., Nishida K., *Biol. Pharm. Bull.*, **36**, 1807–1813 (2013).
158) Fumoto S., Nishi J., Ishii H., Wang X., Miyamoto H., Yoshikawa N., Nakashima M., Nakamura J., Nishida K., *Mol. Pharm.*, **6**, 1170–1179 (2009).
159) Chou T. C., *Pharmacol. Rev.*, **58**, 621–681 (2006).