Nanoparticle-assisted combination therapies for effective cancer treatment

Combination chemotherapy and nanoparticle drug delivery are two areas that have shown significant promise in cancer treatment. Combined therapy of two or more drugs promotes synergism among the different drugs against cancer cells and suppresses drug resistance through distinct mechanisms of action. Nanoparticle drug delivery, on the other hand, enhances therapeutic effectiveness and reduces side effects of the drug payloads by improving their pharmacokinetics. These two active research fields have been recently merged to further improve the efficacy of cancer therapeutics. This review article summarizes the recent efforts in developing nanoparticle platforms to concurrently deliver multiple types of drugs for combination chemotherapy. We also highlight the challenges and design specifications that need to be considered in optimizing nanoparticle-based combination chemotherapy.

The emergence of nanotechnology has made a significant impact on clinical therapeutics in the last two decades. Advances in biocompatible nanoscale drug carriers such as liposomes and polymeric nanparticles have enabled more efficient and safer delivery of a myriad of drugs. Advantages in **nanoparticle drug delivery**, particularly at the systemic level, include longer circulation half-lives, improved **pharmacokinetics** and reduced side effects [1–3]. In cancer treatments, nanoparticles can further rely on the enhanced permeability and retention effect caused by leaky tumor vasculatures for better drug accumulation at the tumor sites [4]. These benefits have made therapeutic nanoparticles a promising candidate to replace traditional chemotherapy, where intravenous injection of toxic agents poses a serious threat to healthy tissues and results in dose-limiting side effects. Currently, several nanoparticle-based chemotherapeutics have emerged on the market, while many are undergoing various stages of clinical or preclinical development [5]. Notable examples of chemotherapeutic nanoparticles include Doxil® (a ~100-nm liposomal formulation of doxorubicin) [6] and Abraxane (a ~130-nm paclitaxel-bound protein particle) [7], both of which are routinely administered as first-line treatments in various cancer types.

In recent years, scientists and engineers have been exploring different approaches to delivering multiple therapeutic agents with a single drug nanocarrier. Such efforts have been motivated by the fact that applying multiple drugs can suppress the notorious phenomenon known as cancer **chemoresistance**, which is accountable for most of the failed cases in cancer therapy. It has been frequently observed that cancer cells show diminishing response over the course of a chemotreatment as they acquire defense mechanisms by overexpressing drug efflux pumps, increasing drug metabolism, enhancing self-repairing ability or expressing altered drug targets [8]. To reduce cancer drug resistance for better therapeutic effectiveness, combination chemotherapy has long been adopted in clinics as a primary cancer treatment regimen. On the one hand, applying multiple drugs with different molecular targets can raise the genetic barriers that need to be overcome for cancer cell mutations, thereby delaying the cancer adaptation process. On the other hand, it has also been demonstrated that multiple drugs targeting the same cellular pathways could function synergistically for higher therapeutic efficacy and higher target selectivity [9]. However, current combination chemotherapies are far from perfect. Varying pharmacokinetics, biodistrubtions and membrane transport properties among different drug molecules make dosing and scheduling optimization extremely difficult. Furthermore, highly potent drug combinations are often associated with more serious side effects. These challenges have driven researchers and clinicians to investigate clever and elegant approaches to incorporating nanotechnology with combination chemotherapy.

This article reviews the latest status of nanoparticle-assisted co-delivery of multiple drugs for **combination therapy**. The focus of

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this paper is distinct from the broader generalization of nanoparticle-based combination therapy, which includes co-administration of multiple different single drug-containing vehicles, as discussed in a recent review by Greco *et al* [10]. We emphasize multidrug-containing nanoparticles over co-delivery of single drug-containing particles because they offer the unique features of vehicle uniformity, ratiometric drug loading and temporal drug release. All of these features carry significant therapeutic implications and will be discussed in detail. In this review, the properties and synthesis of several nanoscale drug carriers including liposomes, polymeric nanoparticles, dendrimers and silica nanoparticles are discussed with an emphasis on the mechanisms through which multidrug co-encapsulation can be achieved. In addition to traditional anticancer drugs, co-delivery of emerging classes of oncological therapeutics such as small interfering RNA (siRNA), antiangiogenic agents and chemosensitizers are briefly reviewed to demonstrate that the nanoparticle platforms are capable of co-delivering a wide range of therapeutic options. Finally, we emphasize the challenges and design specifications that need to be considered for future development of drug-delivery nanoparticles for combination therapies.

Nanoparticle platforms for combination cancer therapy

■ Liposomes

First described in 1965 [11], the liposome is the most established drug-delivery vehicle, with many clinical products to date. Liposomes consist of amphiphilic lipid molecules that assemble into bilayered spherical vesicles. This assembly process usually requires external energy from sonication, homogenization, shaking or heating [12,13]. Phosphatidylethanolamine and phosphatidylcholine are common building blocks of liposomes and cholesterol is frequently incorporated into liposomal membranes to enhance their stability and rigidity. The emergence of 'stealth liposomes,' or polyethyleneglycol (PEG)-coated liposomes, took liposomal drug delivery to a whole new level as they increased the *in vivo* circulation half-life of liposomes from a few hours to approximately 45 h [14]. Currently, liposomal products used for cancer treatment include Doxil [15,16], DaunoXome® [17,18], DepoCyt® [19,20] and ONCO-TCS [21], which are liposomal formulations of doxorubicin, daunorubicin, cytarabine and vincristine, respectively.

Drug encapsulation in liposomes can be achieved by two different methods. First, the drugs can be dissolved in an aqueous solution to hydrate lipid films. This process results in the formation of drug-loaded multilamellar liposomes that can then be extruded through filters with a predetermined pore size to form unilamellar liposomes. Second, unilamellar liposomes are first synthesized and subsequently incubated in an aqueous drug solution. The drug molecules can diffuse passively through the liposomal membranes until the aqueous cavity is saturated. The unloaded drugs will then be removed from the drug-loaded liposome solution through dialysis, column chromatography or centrifugation. Owing to their unique structure, liposomes can simultaneously load hydrophilic drugs in their aqueous core and hydrophobic drugs in their lipid bilayered membrane [22]. This property makes liposomes a highly versatile platform for combination drug delivery.

An early attempt to create dual drug-loaded liposomes by Agrawal *et al.* in 2005 highlights both the promises and challenges in combinatorial drug delivery using single nanoparticles [23]. In the study, the authors encapsulated two antileukemia drugs, 6-mercaptopurine and daunorubicin, into a single liposome and examined their loading efficiency as well as their *in vitro* cytotoxicity. The two drugs have dissimilar working mechanisms as 6-mercaptopurine hinders purine biosynthesis and daunorubicin inhibits DNA topoisomerase II [24]. The dual drug-loaded liposomes exhibited higher cytotoxicity against Jurkat and Hut 76 T-cell lymphoma as compared with monodrug-loaded liposomes containing either 6-mercaptopurine or daunorubicin, suggesting that the combination of these two mechanistically different drugs can generate higher therapeutic efficacy. However, the study was limited by the low entrapment efficiency of 6-mercaptopurine owing to its poor solubility in either aqueous or lipid phases. A maximum of 1.5% of 6-mercaptopurine was loaded into the liposomes, while the lipophilic daunorubicin was readily partitioned into the lipid membranes with an encapsulation efficiency of up to 55% [23]. The results of this study demonstrate the feasibility of nanoparticle-based dual drug delivery and inspire increasing efforts on dosing control in multidrug co-encapsulation.

More recently, maturation in liposome synthesis and drug-encapsulation processes have yielded precise control over combinatorial

drug dosing in liposomes. By adjusting the lipid composition, drug concentration during lipid film hydration, liposome incubation process and incubation time, Mayer *et al.* were able to load several combinations of drugs into liposomes at comparable and adjustable molar ratios [25]. *In vivo* pharmacological studies with these liposomes revealed that the initial loading molar ratios of different drugs were well maintained in the circulation for up to 24 h. This work makes a significant stride in bridging the gap between *in vitro* design and characterization and *in vivo* oncological evaluations. It has been well documented in *in vitro* studies that the molar ratio governs whether two drugs can act synergistically, additively or antagonistically [9,26–28]. For instance, the combination of camptothecin and doxorubicin shows synergistic activity against glioma cells at a molar ratio of 1.5:1 and strong antagonism at 5:1 [26]. However, in clinical studies drug ratio has often been an afterthought and different drugs are administered based on their maximal tolerated dose. By overcoming the dissimilar pharmacokinetics of different drug molecules, ratiometric liposomal formulations enable simultaneous delivery of multiple drugs to the target site at a predetermined and optimal molar ratio. This technology has yielded several products that are currently in clinical trials. For example, CPX-351 is a 5:1 cytarabine and daunorubicin dual drug-loaded liposome that is currently under Phase II clinical trial for the treatment of acute myeloid leukemia [29]. In murine models bearing HL-60B human leukemia cells, administration of CPX-351 extended the median survival time to 43 days from the 30 days of saline-treated mice. In comparison, ratio-matched free-drug cocktail treatment showed no increase in median survival time compared with saline even at 1.5-fold the dosage of CPX-351 [29]. Moreover, CPX-1, a 1:1 irinotecan and floxouridine liposome currently under Phase II trial for colorectal cancer treatment, also exhibited superior anticancer activity in various human tumor xenograft murine models compared with liposomal irinotecan or liposomal floxouridine alone and free-drug cocktail treatment [30]. It is also worth noting that liposomal co-delivery of irinotecan and floxuridine at an antagonistic ratio showed a poorer response compared with liposomal irinotecan, suggesting that the drug-ratio effect commonly observed *in vitro* can be faithfully translated to *in vivo* by liposomal

co-encapsulation of multiple drugs. These liposomal platforms could bring a paradigm shift in clinical cancer treatment by enabling dosage optimization in combination chemotherapy.

In addition to delivering the aforementioned drug combinations, liposomes can also deliver chemodrugs with chemosensitizers to address multidrug resistance (MDR) associated with the overexpression of P-glycoproteins (Pgp) [31]. For instance, Wu *et al.* synthesized a transferrin-conjugated liposome co-encapsulating doxorubicin and a potent Pgp inhibitor, verapamil, and examined its efficacy against doxorubicin-resistant K562 cells [32]. Compared with the liposomal doxorubicin, which showed an IC_{50} of 11.4 µM against these doxorubicin-resistant cells, the liposomes co-encapsulating doxorubicin and verapamil showed a threefold increase in toxicity with an IC_{50} of 4.18 µM. Since systemic injection of verapamil can cause serious cardiotoxicity, liposomal delivery of verapamil together with chemodrugs presents a promising approach to reversing cancer drug resistance and minimizing verapamil-related side effects [33,34].

Another example of liposome-based combination therapy is the co-delivery of siRNA and chemodrugs. siRNA is an emerging class of cancer therapeutics that interferes with gene expression by targeting specific mRNA sequences. A recent study by Saad *et al.* developed a liposome system to deliver BCL2 (a protein responsible for anti-apoptotic cellular defense) and MRP1 (a multidrug resistance-associated protein) targeted siRNA in combination with doxorubicin against human H69AR lung cancer cells [35]. A positively charged 1,2-dioleoyl-3-trimethylammonium-propane is used to construct a cationic liposome, which was then loaded with doxorubicin through an incubation method and subsequently incubated with siRNA. The liposome–siRNA complex formed through electrostatic interaction as the negatively charged phosphate groups on siRNA molecules bind to the positively charged liposomal surface. An *in vitro* cytotoxicity study showed considerable reversal of cellular resistance in MDR lung cancer cells. Although the study is at a preliminary stage, it demonstrates the versatility of liposomes as a multidrug nanocarrier.

Various encapsulation schemes of liposome-based combinatorial drug delivery are illustrated in **FIGURE 1**. **TABLE 1** provides a summary of liposome-assisted combination therapies for cancer treatment.
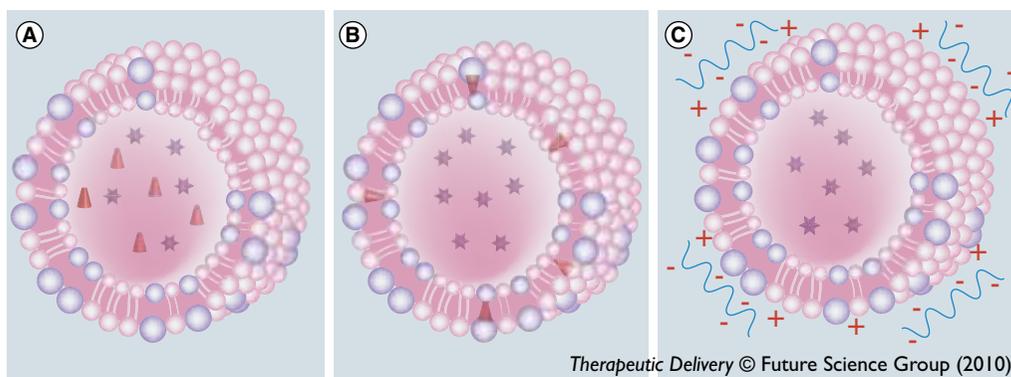
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Figure 1. Liposomal platforms for co-delivery of multiple drugs. (A) Co-encapsulation of multiple hydrophilic drugs (cones and stars); **(B)** co-encapsulation of lipophilic (cones) and hydrophilic drugs (stars) and **(C)** co-delivery of hydrophilic drugs (stars) and oligonucleotide drugs such as siRNA (curved lines).

■ Polymeric nanoparticles

Advances in biomaterials research have led to the emergence of biocompatible and biodegradable polymeric nanoparticles for drug-delivery applications. Several synthetic polymers approved by the US FDA such as poly(lactic-co-glycolic acid) (PLGA) and polycaprolactone (PCL) and several natural polymers such as chitosan and polysaccharides have been investigated extensively for nanoparticle synthesis [36–41]. Compared with liposomes, polymeric nanoparticles generally have higher stability, sharper size distribution, more tunable physicochemical properties, sustained and more controllable drug-release profiles, and higher loading capacity for poorly water soluble drugs. The polymer platform also offers higher synthetic freedom that allows particles to be tailored for specific needs. Owing to these unique characteristics, polymeric nanoparticles have attracted tremendous interests from academia, industry and clinic, although they are still in a relatively early stage of development.

Polymeric nanoparticles typically consist of amphiphilic diblock copolymers that self assemble into nanoparticles in aqueous solutions. For *in vivo* drug delivery, PLGA and PEG are popular choices for the hydrophobic and the hydrophilic block, respectively, as PLGA can hydrolyze into lactic acid and PEG can significantly reduce nonspecific cellular uptake by forming a stealth layer [42,43]. Drug encapsulation is typically achieved by mixing the drugs with the polymer solutions during particle preparation process. For instance, in nanoparticle synthesis through solvent displacement technique, a water-miscible solvent such as acetonitrile is used to dissolve the hydrophobic drugs together with the diblock copolymers. The solution is subsequently mixed with water. The organic solvent diffuses into the aqueous phases and eventually evaporates ,and the hydrophobic polymers self assemble to form nanoparticles with drugs encapsulated inside. Although polymeric nanoparticles are most suitable for delivering hydrophobic drugs, several reports have shown

Table 1. Liposomes for combination cancer therapy.				
Formulation	**Drugs**	**Indication**	**Status**	**Ref.**
CPX-351	5:1 cytarabine and daunorubicin	Acute myeloid leukemia	Phase II	[29]
CPX-1	1:1 irinotecan and floxuridine	Colorectal cancer	Phase II	[30,72]
CPX-571	7:1 irinotecan and cisplatin	Small-cell lung cancer	*In vivo*	[73]
Liposomes co-encapsulating 6-mercaptopurine and daunorubicin	6-mercaptopurine and daunorubicin	Acute lymphocytic leukemia	*In vitro*	[23]
Liposome co-encapsulating quercetin and vincristine	1:2 quercetin and vincristine	Breast cancers	*In vitro*	[74]
Cationic liposome co-encapsulating siRNA and doxorubicin	Doxorubicin, MRP1-targeted siRNA and BCL2-targeted siRNA	Lung cancer	*In vitro*	[35]
Transferrin-conjugated liposomes co-encapsulating doxorubicin and verapamil	Doxorubicin and verapamil	Leukemia	*In vitro*	[32]

success in encapsulating hydrophilic drugs through surface attachment or polymer–drug conjugation methods [36,44–46].

Many approaches have been taken to co-encapsulate multiple therapeutic agents into a single polymeric nanoparticle. Presently, these approaches can be divided into three major categories, as follows:

- Directly encapsulating multiple drugs into the hydrophobic polymeric core;

- Incorporating an additional media compartment to the nanoparticle, usually on the particle surface, to create a separate partition for drug loading;

- Covalently conjugating multiple drugs to the polymer backbone before nanoparticle synthesis.

In the first approach, multiple therapeutic agents are mixed with the polymer solution during particle synthesis. This approach is widely adopted for the co-delivery of anticancer drugs and chemomodulators. One example is the co-encapsulation of verapamil together with vincristine to increase the chemosensitivity in drug resistant cancer cells. The dual drug-loaded nanoparticles were prepared by mixing PLGA, verapamil and vincristine in acetone–dichloromethane solution at predetermined concentrations before solvent displacement and particle precipitation [47]. Another example can be found in the co-encapsulation of doxorubicin and cyclosporin A using polyalkylcyanoacrylate nanoparticles, in which the hydrophobic cyclosporin A (a potent inhibitor of Pgp) was added together with doxorubicin to the polymerization medium to achieve dual drug loading [48]. In both studies, the dual drug-loaded nanoparticles showed markedly higher *in vitro* cytotoxicity against resistant cancer cell lines, providing a viable solution to the MDR effect of cancer cells that has plagued many cancer treatments.

While the first co-encapsulation approach is easy to implement, it offers little control over the release kinetics of different drugs and is inefficient in loading hydrophilic compounds. To address these issues, a multicompartment approach has been employed by taking advantage of the highly functionalizable surface of polymeric nanoparticles. Zhang *et al.* reported an aptamer–nanoparticle bioconjugate that could co-deliver hydrophobic and hydrophilic drugs. In their study, the PLGA nanoparticles loaded with a hydrophobic chemodrug, docetaxel, were surface modified with oligonucleotides that serve both as targeting

ligands and as intercalation sites for a hydrophilic chemodrug, doxorubicin [36]. The conjugate not only demonstrated targeted co-delivery of docetaxel and doxorubicin but also exhibited differential drug release kinetics. A drug release study revealed that the intercalated doxorubicin was released faster than the physically entrapped docetaxel. At the 6 h mark, approximately 80% of the doxorubicin and only approximately 45% of docetaxel were released. This feature carries significant clinical implications as drug sequencing and scheduling are crucial parameters in combination chemotherapy [9,49,50]. The ability to modulate the release profile of different drugs independently enables more intricate designs that may further improve therapeutic efficacy of drug combinations.

The advantage of temporal control on drug release is exemplified in another multipartitioned dual drug nanoparticle, which combines anti-angiogenic agents with doxorubicin. Sengupta *et al.* synthesized a nanocell consisting of a PLGA core and a lipid envelope to demonstrate the potential of differential drug release in combination anticancer therapy [44]. In this nanoparticle-based dual drug-delivery system, combretastatin, which causes vascular shutdown inside tumors, is encapsulated in the lipid layer. Doxorubicin, on the other hand, is covalently conjugated to PLGA polymers and loaded into the polymeric core. Since doxorubicin is linked to the PLGA polymer, its release was determined by the hydrolytic degradation rate of the polymer and was much slower than the release of combretastatin from the lipid envelope. Upon tumor accumulation, these nanocells first release the antiangiogenic agents to close off the tumor vessels, creating a closed pocket containing the tumor and the doxorubicin-loaded nanoparticles. The subsequent release of doxorubicin can then exterminate the cancer cells focally without being diluted by the blood circulation. The polymeric nanocell was compared with liposomal co-encapsulation of combretastatin and doxorubicin, which lacks the differential drug release kinetics. In murine models bearing Lewis lung carcinoma and B16/F10 melanoma, the nanocell system resulted in better tumor reduction, longer median survival time, as well as lower systemic toxicity. The study demonstrates that nanoparticle-based combination therapy can go beyond simply bringing multiple drugs to the tumor target. By integrating pharmacology with synthetic chemistry through elegant engineering designs, innovative treatment options can be realized.

Advances in polymer chemistry have led to a third approach to co-encapsulating multiple therapeutic agents into a single polymeric nanoparticle. In this approach, polymer–drug conjugates, in which multiple types of drugs can be attached to a single polymer chain, are synthesized prior to nanoparticle synthesis. For instance, doxorubicin and wortmannin (a potent kinase inhibitor) have been concurrently conjugated to PEG–poly(aspartate hydrazide) block copolymers through an acid-labile hydrazone bond [51]. The pH-sensitive hydrazone linker is an important feature in this conjugate as it allows the release of the functional moieties by undergoing rapid hydrolysis in the acidic endosomal and lysosomal environments. What distinguishes this conjugation approach from other drug encapsulation methods is its ability to precisely control the molar ratios of different drugs as it bypasses the complex nature of drug–drug and drug–polymer interactions involved in the physical drug encapsulation techniques. It has been reported that the molar ratios between doxorubicin and wortmannin could be precisely tuned simply by varying the drug content during the conjugate synthesis process.

More recently, temporal control on drug release has been implemented on polymer–drug conjugates by using peptide linkers that are susceptible to intracellular proteases. In a study by Lammers et al., two chemotherapeutic agents, gemcitabine and doxorubicin, have been conjugated to HPMA monomers via a Gly-Phe-Leu-Gly peptide sequence [45]. This peptide sequence is a known substrate to a lysosomal cysteine protease, cathepsin B. In a drug-release study, where the conjugates were incubated with cathepsin B at pH 6, significantly differential release kinetics were observed between the two drugs. While 100% of gemcitabine was released in less than 10 h, 70% of doxorubicin remained attached to the polymer after 30 h. The drastically slower doxorubicin release profile was attributed to the bulky doxorubicin structure, which might hinder the cathepsin activity by blocking the peptide substrate. Although it is unknown whether a sequential release of gemcitabine and doxorubicin will benefit the patients, this study sets a foundation for other drug combinations. It is expected that similar peptide linkers with different specificity toward proteolytic enzymes or other environment-sensitive bonds with different reaction kinetics to intracellular stimuli can be utilized to fine tune the drug-release kinetics from multidrug-loaded nanoparticles.

It is worth noting that polymeric nanoparticle platforms have rapidly evolved as polymer chemists continue to explore novel approaches to synthesizing polymer–drug conjugates. Tong et al., for instance, demonstrated a macromolecular polymerization technique in which the drug molecule itself initiates the ring opening polymerization of cyclic lactones. In the study, a zinc- and magnesium-based β-diiminate complex catalyst is used to polymerize chemodrugs containing a hydroxyl group [52–54]. It is the authors' opinion that these fledging polymerization techniques could potentially pave the road to better nanoparticle-based combinatorial drug delivery by increasing drug loading yields and improving dosing control in multidrug co-encapsulation.

The aforementioned approaches to co-encapsulating multiple drugs into polymeric nanoparticles are illustrated in **Figure 2**. **Table 2** summarizes the recent developments of polymeric nanoparticle-assisted combination therapies for cancer treatment.

■ Dendrimers

Dendrimers are a novel class of nanoparticles that are emerging as a drug-delivery vehicle for cancer therapeutics. They are highly branched globular macromolecules that are synthesized in a stepwise and iterative fashion. The structure of dendrimers can be defined by an initiator core, layers of branched repeating units and functional end groups on the outermost layer. The unique properties of dendrimers make them a desirable platform for concurrent delivery of water soluble and insoluble drugs. For instance, the hydrophobic core contains a cavity that can encapsulate hydrophobic drugs. The multivalent surface, on the other hand, can be conjugated with hydrophilic drugs **(Figure 3A)**. Even though dendrimers have not attracted as much attention as liposomes and polymeric nanoparticles, several attempts have been made to deliver multiple therapeutic drugs simultaneously using a dendritic platform. By taking advantage of the dendrimer structure, Tekade et al. co-encapsulated methotrexate (a hydrophobic chemotherapeutic agent) and all-trans retinoic acid (a hydrophilic compound with mild anticancer activity) in a generation 5 poly(propyleneimine) dendrimer [55]. In this dual drug-loaded dendrimer formulation, methotrexate was loaded into the hydrophobic cavity whereas the small retinoic acids were lodged inside the small voids between branching clefts. Electrostatic interactions between the
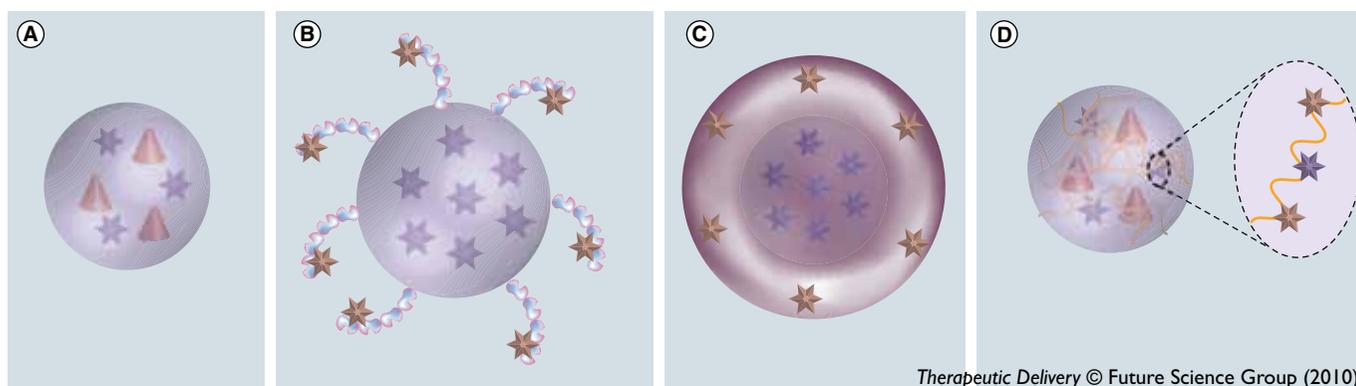
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Figure 2. Polymeric nanoparticle platforms for co-delivery of multiple drugs. (A) Bare polymeric nanoparticle for co-encapsulation of multiple hydrophobic drugs (cones and stars); **(B)** oligonucleotides modified polymeric nanoparticle with hydrophobic drugs (interior stars) entrapped inside the particle and hydrophilic drugs intercalated in the oligonucleotides (exterior stars); **(C)** lipid-coated polymeric nanoparticle with drugs entrapped in the polymeric core (interior stars) and lipid envelope (exterior stars) respectively; and **(D)** polymeric nanoparticle with multiple drugs covalently conjugated to the polymer chains (cones and stars).

carboxyl groups of the drug molecules and the amine terminal groups on the dendrimers helped to stabilize the loaded drugs and also gave rise to a pH-dependent drug-release profile. Under acidic condition, deprotonation of the carboxylic group and conformational change of the dentritic structure accelerated the release of drugs from the dendrimer particles. Under neutral and alkaline pH, however, much slower release kinetics were observed. The pH-triggered drug-release property could reduce systemic toxicity by minimizing premature drug leakage during the circulation period. Only upon endocytic uptake by the target cells would the vehicle releases its drug payloads.

Dendrimers can also carry siRNA through surface electrostatic interactions. A generation-3 nanoglobular dendrimer (poly-L-lysine)

octa(3-aminopropyl) silsesquioxane) surface modified with a tumor-targeting peptide, c(RGDFK), has been reported to carry both doxorubicin and siRNA for targeted combination therapy [56]. These siRNA–dendrimer complexes were readily internalized by U-87 glioblastoma cells via receptor-mediated endocytosis and showed significant gene-silencing activity. Other examples of dendrimer-based combination cancer therapy are summarized in TABLE 3.

■ **Other nanoparticles**
The surging interest in nanotechnology has yielded a variety of nanoparticulate systems in addition to the aforementioned nanoparticle types. Metallic nanoparticles, silica nanoparticles

Table 2. Polymeric nanoparticles and polymer–drug conjugates for combination cancer therapy.

| Formulation	Drugs	Indication	Status	Ref.
HPMA–Gem–Dox	Gemcitabine and doxorubicin	Prostate cancer and various cancer types	In vivo	[45]
Poly(ethylene glycol)–poly(aspartate hydrazide) block copolymers–Dox–WOR	Doxorubicin and phosphatidylinositol-3 kinase inhibitor (Wor)	Breast cancer and various cancer types	In vitro	[51]
Combretastatin–doxorubicin nanocell	Combretastatin and doxorubicin	Lung carcinoma, melanoma and various cancer types	In vivo	[44]
Cationic core-shell nanoparticles.	Paclitaxel and Bcl-2-targeted siRNA	Breast cancer	In vitro	[75]
PDMAEMA–PCL–PDMAEMA-based cationic micelles	Paclitaxel and VEGF siRNA	Prostate cancer and various cancer types	In vitro	[76]
Nanoparticle–aptamer bioconjugates	Doxorubicin and docetaxel	Prostate cancer and various cancer types	In vitro	[36]
Poly(lactic-co-glycolic acid) nanoparticle co-encapsulating vincristine and verapamil	Vincristine and verapamil	Breast cancer	In vitro	[47]
Polyalkylcyanoacrylate nanoparticles co-encapsulating doxorubicin and cyclosporin A	Doxorubicin and cyclosporin A	Various cancer types	In vitro	[48]
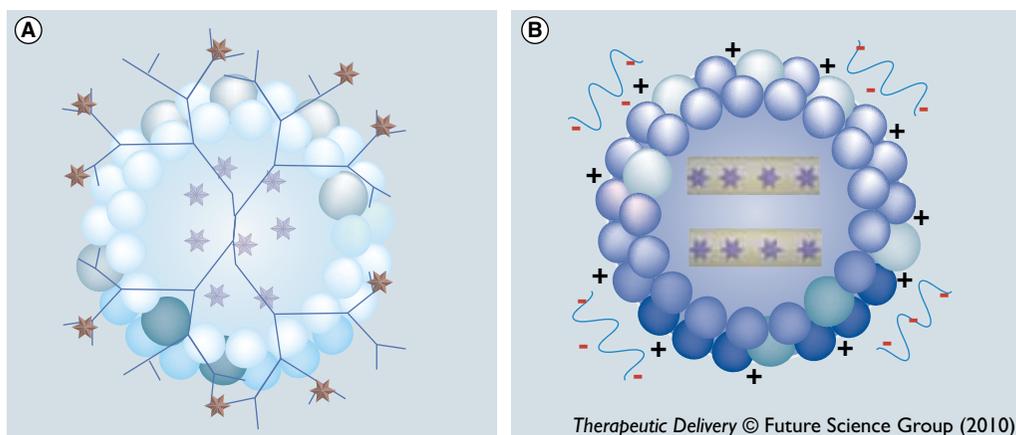
Figure 3. Dendrimer and mesoporous silica nanoparticle platforms. (A) Dendrimer carrying multiple chemotherapy drugs (interior and exterior stars) and **(B)** mesoporous silica nanoparticle carrying small molecule chemotherapy drugs (stars) and oligonucleotide drugs such as siRNA (curved lines).

and carbon nanotubes, for instance, have all been investigated as drug carriers for the treatment of various diseases [57–61]. However, these nanoparticle platforms are generally less functionalizable and, thus, have not generated much excitement as multidrug-delivery platforms. To address the lack of synthetic flexibility in inorganic nanoparticles, Chen *et al.* synthesized a hybrid structure combining silica nanoparticles with dendrimers and demonstrated its ability to co-deliver doxorubicin and siRNA [62]. To synthesize this silica-based dual drug-delivery platform, a mesoporous nanoparticle was first loaded with doxorubicin through passive diffusion. The silica particle was then modified with amine-terminated second-generation polyamidoamine dendrimers. As described earlier, the cationic dendrimer surface can readily bind to the negatively charged phosphate backbone of the siRNA molecules. *In vitro* studies showed that the resulting nanoparticle complex can simultaneously deliver doxorubicin and siRNA to cancer cells and enhance the efficacy of chemotherapy. This study indicates that two distinct nanoparticulate systems can be combined together to form a more potent nanoparticle platform. **Figure 3B** illustrates a silica nanoparticle-based dual drug-delivery platform.

Challenges & design specifications

In this section, we highlight the challenges and factors that need to be considered when one designs drug-delivery nanoparticles for combination therapies.

■ Ratiometric drug loading

One of the biggest motivations behind nanoparticle-assisted combination chemotherapy is the ability to unify the pharmacokinetics of different drugs by simultaneously delivering multiple therapeutic agents to the target site. This would minimize the gap between *in vitro* and *in vivo* studies and enhance the possibility of bench-to-bedside translation. The therapeutic efficacy of multiple-drug-loaded nanoparticles

Table 3. Dendrimers and other nanoparticles for combination cancer therapy.				
Formulation	**Drugs**	**Indication**	**Status**	**Ref.**
Generation-3 poly(L-lysine) octa(3-aminopropyl)silsesquioxane dendrimer	Doxorubicin and siRNA	Glioblastoma	*In vitro*	[56]
Generation-5 poly(propyleneimine) dendrimer with ethylenediamine core	Methotrexate and all-*trans* retinoic acid	Leukemia	*In vitro*	[77]
Generation-4 polyamidoamine dendrimers	Methotrexate and all-*trans* retinoic acid	Leukemia	*In vitro*	[55]
Oil nanoemulsion coencapsulating paclitaxel and curcumin	Paclitaxel and curcumin	Ovarian cancer	*In vitro*	[78]
Mesoporous silica nanoparticles	Doxorubicin and Bcl2-targeted siRNA	Ovarian cancer	*In vitro*	[62]

would be greatly compromised if drug loading of different drugs cannot be precisely controlled. This is especially an issue for passive drug loading in a delivery vehicle such as polymeric nanoparticles because drug–drug and drug–polymer interactions often cause unpredictable batch-to-batch inconsistency of drug-loading yields.

■ **Temporal drug release**

Sequential delivery and scheduling of combinatorial drugs are important parameters that determine **drug synergism** and side effects of many drug combinations. For instance, it has been reported that treating estrogen receptor-positive breast cancer cells with ibandronate followed by tamoxifen was approximately 1.6-fold more effective than the reverse treatment sequence or simultaneous administration in terms of prohibiting cell growth [63]. A drug-delivery system that can control the release sequence of its drug payloads will provide better tailored cancer treatment, which holds great promise to overcome cancer drug resistance by more effectively targeting molecular pathways of cancer cells. Moreover, temporal drug release may also promote the transport and penetration of therapeutic nanoparticles to deep tissue of solid tumors through a tumor priming mechanism. The first released drug induces partial cancer cell apoptosis and expands the interstitial space of solid tumors and, thus, the nanoparticles can diffuse into the deep tumor tissue and release the second drug for more effective cancer treatment. More detailed description of tumor priming can be found in some recent reports by Lu *et al.* [64,65].

■ **Targeted delivery**

As therapeutic nanoparticles co-encapsulating multiple types of drugs are more potent against cancer cells, they are also more likely to inflict collateral damage on healthy tissues. Targeted delivery toward the tumor is therefore an important element in the development of nanoparticle-based combination therapy. Even though nanoparticles can passively accumulate at the tumor site through enhanced permeability and retention effects, active targeting can further aid the process. Liposomes, polymeric nanoparticles and dendrimers all contain surface functional groups that can be conjugated to targeting ligands for tumor-specific drug delivery. Examples of targeting ligands for nanoparticle delivery include peptides (RGD and Lyp1) [66], oligonucleotides (aptamers) [67], antibodies [68,69] and antibody variants (single-chain variable fragments and diabodies) [70,71].

■ **Manufacturability**

Last but not least, manufacturability is an important factor that needs to be considered when designing multiple drug co-delivery nanoparticles. A complex synthetic scheme would increase particle heterogeneity and reduce cost effectiveness, thereby hindering clinical translation. A simple and straightforward preparation process will make large-scale fabrication of multiple drug loaded nanoparticles practically possible.

Conclusion

In summary, this review has shown a range of nanoparticle platforms for combinatorial drug delivery. Liposomes, polymeric nanoparticles, dendrimers and silica nanoparticles have been demonstrated to carry a variety of anticancer agents including cytotoxic drugs, chemomodulators, siRNA and antiangiogenic agents. Precise control over the particle composition and preparation has enabled ratiometric drug loading and temporal drug release, both of which carry significant clinical implications in cancer treatments.

Future perspective

Nanoparticle drug delivery has yielded an unprecedented level of control over the pharmacokinetics of chemotherpeutic agents. Recent development in nanoparticle-based combination therapy have shown several unique features that are untenable in traditional chemotherapy. Drug combinations can now be optimized and cleverly delivered in a more effective way. With a growing alliance between oncologists and engineers, we envision that more therapeutic nanoparticles containing multiple drugs with precise drug dosage and release profiles will be developed to treat various types of cancer. In addition, emerging techniques in drug–polymer conjugations and nanomaterials engineering will continue to expand the nanoparticle platforms on which better therapeutic regimens can be designed.

Key Term

Drug synergism: Enhancement or magnification of drug's effects as a result of the presence of two or more drugs within the system.

Executive summary

- Molar ratio and sequencing in drug combinations greatly influence their therapeutic efficacies. These parameters have been difficult to control *in vivo* owing to the varying pharmacokinetics among different drug molecules.

- Ratiometric drug loading has been achieved in liposomes by controlling the lipid composition and liposome preparation. The molar ratio between two drugs can be maintained *in vivo*.

- Polymeric nanoparticles have demonstrated differential drug release profiles through multicompartalization or through drug-polymer conjugates with environment-sensitive linkers.

- Dendrimers are suitable for co-delivery of water soluble and insoluble drugs as they typically contain a hydrophobic core and hydrophilic branches.

- Modification of inorganic nanoparticles with polymers can improve their synthetic flexibility for multidrug encapsulation.

- A few key factors need to be considered for designing new drug delivery nanoparticles for combination therapies, including ratiometric drug loading, temporal drug release, targeted delivery and manufacturability of the therapeutic nanoparticles.

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Papers of special note have been highlighted as:
- of interest
- ■ ■ of considerable interest

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