Current development in nanoformulations of docetaxel

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Introduction: Docetaxel (DTX) has been proven as one of the most important cytotoxic agents, and its clinical efcacy against many cancers is superior to paclitaxel. DTX in commercial formulation contains the non-ionic surfactant Tween 80 (polysorbate 80) and 13% ethanol; the side effects caused by DTX and the solvent have considerably limited its clinical use. In recent decades, the emergence of nanoformulations provides new modes of actions in DTX. Many nano-sized carriers can help DTX transport through leaky tumor capillary fenestrations into the tumor cells. Moreover, these particles can be modified for binding to specific sites such as cancer cell membranes, cytoplasmic or nuclear receptors.

Areas covered: The authors focus on nanoformulations related to DTX delivery, covering their preparation, physicochemical properties and the *in vitro* and *in vivo* actions against tumor cells. The challenges involved in the development of nanoformulations for DTX are also discussed.

Expert opinion: Although nanoformulations such as liposome, micelle, nanoparticle, nanoemulsion greatly improve the solubility, activity and distribution of DTX *in vivo,* significant hurdles remain concerning aspects of nanoformulations such as quality control, physicochemical stability, storage conditions, large-scale production and controlled manufacture technology, *in vivo* metabolism, excretion, acute and chronic toxicity, etc. In-depth studies in these areas are essential to making DTX nanoformulations applicable in clinic and commercially available viable.

Keywords: docetaxel, liposome, micelle, nanoemulsion, nanoparticle

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1. Introduction

Docetaxel (DTX) is a semi-synthetic analog of paclitaxel which is an extract from a rare Pacific yew tree *Taxus brevifolia* [1]. DTX is more water-soluble than paclitaxel owing to its chemical structure in which there is a tertbutyl carbamate ester in its phenylpropionate side chain and a hydroxyl functional group on carbon 10 (**Figure 1**). By binding to and stabilizing tubulin, DTX prevents physiological microtubule depolymerization and disassembly, leading to cell cycle arrest at the G2/M phase and cell death. DTX also inhibits expression of the anti-apoptotic gene Bcl2 and promotes the expression of the cell cycle inhibitor p27 [2-4]. These effects confer DTX antitumor activities against a broad range of tumors [5], including breast, non-small cell lung cancer, ovarian, gastric and prostate carcinomas [6-10].

However, DTX has not gained clinical use owing to its poor solubility, low selective distribution and fast elimination. In addition, currently available commercial formulations of DTX (Taxotere® and Duopafei®) have been reported to cause serious side effects which include neutropenia, musculoskeletal toxicity, peripheral neuropathy and hypersensitivity reactions, attributable to either DTX itself or the solvent (polysorbate 80) [11,12]. Therefore, overcoming the side effects of DTX and improving its anticancer effects have been a focus of studies of nanocarriers.

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Article highlights.

- Docetaxel (DTX) is one of the most important cytotoxic agents, and its clinical efficacy against many cancers is even better than paclitaxel.
- Commercial formulations of DTX have been reported with serious side effects due to either DTX itself or the solvent.
- Nanotechnology has great potential for DTX delivery due to its advantages such as small size, drug solubilization, controlled drug release, escaping from reticuloendothelial system (RES) uptake and tumor targeting by enhanced permeability and retention (EPR) effect. Many nano-sized carriers can help DTX transport through leaky tumor capillary fenestrations into the tumor cells.
- Novel strategies (e.g., antibody- or peptide-targeted delivery) for active targeting have been carried out, expecting to enhance the anticancer efficiency while decreasing the toxicity to normal tissues.
- Many relative problems such as the quality control, physicochemical stability, storage conditions, large-scale and controlled manufacture technology, *in vivo* metabolism, excretion, acute and chronic toxicity of nanoformulations loading DTX have not been deeply and extensively studied in the present available reports.

This box summarizes key points contained in the article.

Nanocarriers for drug delivery are architecturally designed varying in sizes, shapes, materials and structures. Different nanocarriers have their own unique characteristics that play distinctive important roles in drug delivery [13]. Delivery of nanocarriers for DTX can be accomplished through an active or passive process. Passive delivery refers to particle transportations into the tumor cells and interstitium by passive diffusion and convection through leaky tumor capillary fenestrations [14]. Selective accumulation of particles and drug could be achieved through enhanced permeability and retention (EPR) effect. By contrast, active delivery involves targeting specific sites for drug delivery based on molecular interactions. For instance, a ligand can be coupled to a particle to capture its receptor, and an antibody can be attached to a particle to recognize a specific antigen in the cells. Therefore, a targeted drug delivery system may improve the anticancer efficacy of DTX, reduce its toxicity on normal cells, and slower the development of multidrug resistance (MDR) resulting from continued exposure of tissues to sublethal doses of chemotherapy [15].

Up to now, several nanoparticles (NPs) for drug delivery are approved by the Food and Drug Administration (FDA) and available for cancer treatment, including pegylated liposomal doxorubicin and albumin-bound paclitaxel NP Abraxane [16-21], and increased number of nanoformulations, such as NK105 and NK012 which deliver paclitaxel and SN-38, respectively, are in advanced clinical testing for cancer therapy [22,23]. However, the nanoformulations for DTX are still limited. This review provides an overview of the current development in the DTX-loaded nanocarriers.

2. Liposomes

Liposomes are composed of one or more phospholipid bilayers enclosing an aqueous phase, and are thus classified as either unilamellar or multilamellar liposomes according to the number of lipid bilayers. Water-soluble drugs can be encapsulated within the aqueous compartments, while lipophilic or amphiphilic compounds can be entrapped between the lipid bilayers. In recent years, great progress in the technology of liposome has been made, and a simple model of multifunctional liposome is presented in **Figure 2**. In **Table 1**, important data (both *in vitro* and in *vivo*) of several advanced liposomes have been summarized, and the results of this research are discussed in this section. A few liposomal DTX formulations are being tested in preclinical or clinical trials. A novel proprietary delivery system of DTX, liposome-encapsulated DTX (LE-DT), developed by NeoPharm, Inc., has been investigated in Phase I in which the LE-DT was evaluated for the maximum tolerated dose, pharmacokinetic, anti-tumor effects and dose-limiting toxicity in patients with advanced solid tumors. A non-pegylated liposomal DTX is in Phase II as this review is being prepared. Its safety, primarily cardiac safety, as well as the efficacy for the treatment of locally advanced or metastatic HER2/neu positive breast cancer in patients not previously received chemotherapy for metastatic cancer are being assessed.

2.1 PEG-modified liposomes

Increasing evidence has suggested that when the drug is incorporated into liposomes, its pharmacokinetics may be completely altered [24,25]. Zhao *et al.* [26] prepared DTX-loaded liposomes by solid dispersion-effervescent technique and investigated their pharmacokinetics and tissue distributions in mice. The results showed that liposomes greatly increased drug accumulation in the lung, but not in the heart, kidney, stomach or brain. So it could be concluded that the DTX liposomes were useful for treatment of lung cancer with reduced toxicity to other tissues and improved therapeutic index. Muthu *et al.* [27] used solvent injection method to prepare conventional (non-coated), 1,2-distearoyl-phosphatidylethanolamine-methyl-polyethyleneglycol conjugate-2000 (DSPE-mPEG$_{2000}$)-coated (PEG-coated), and D-α-tocopheryl polyethylene glycol 1000 succinate monoester (TPGS) (PEG$_{1000}$)-coated liposomes, respectively. The TPGS-coated liposomes showed better controlled release property and higher encapsulation efficiency than the PEG-coated liposomes and the conventional liposomes. Cellular uptake study showed that cellular uptake by C6 glioma brain cancer cells incubated with TPGS-coated liposomes was significantly higher compared with those incubated with conventional liposomes and the PEG-coated liposomes ($p < 0.05$). This is likely attributable to the capability of TPGS to enhance the absorption of the liposomes. Cytotoxicity of liposomes as expressed in the IC_{50} values was 37.04 ± 1.05, 31.04 ± 0.75, 7.70 ± 0.22 and 5.93 ± 0.57 μg/ml, for the commercial Taxotere, the conventional-,

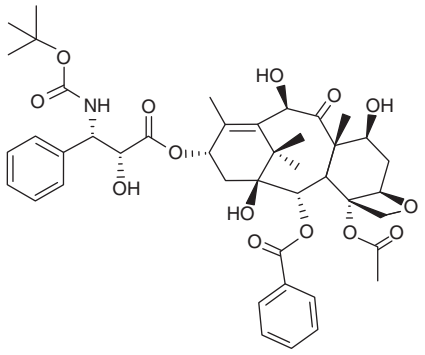

Figure 1. Structure of DTX.

PEG-coated and TPGS-coated liposomes, respectively after 24 h incubation in C6 glioma cells. The results showed that TPGS-coated liposomes exhibited higher cytotoxicity.

2.2 TPGS-modified liposomes for DTX

TPGS is a PEGylated vitamin E. It possesses capabilities of enhancing hydrophobic drugs' solubility, inhibiting P-glycoprotein (P-gp)-mediated MDR, and increasing the oral bioavailability of anti-cancer drugs. These qualities make TPGS an excellent and effective surfactant [28,29]. Muthu *et al.* [30] prepared folate receptor targeting TPGS-coated theranostic liposomes for DTX and quantum dots (QDs) by the solvent injection method. Confocal laser scanning microscopy (CLSM) was used to visualize the targeting effect of the multifunctional liposomes (DTX-QDFA) in MCF-7 cells. Through the confocal images, it was easy to find that, when compared with non-targeting liposomes (DTX-QD), DTX-QDFA was more conducive to increase cellular uptake by MCF-7 cells. In the *in vitro* cytotoxicity study, after 24 h incubation in MCF-7 cells, the IC_{50} value of DTX-QDFA was 0.23 ± 0.05 μg/ml which is greatly smaller than those for Taxotere (9.54 ± 0.76 μg/ml) and DTX-QD (1.56 ± 0.19 μg/ml). However, when administrated DTX-QDFA with 1 mM free folate, the IC_{50} value was greatly increased (1.10 ± 0.21 μg/ml), such reversal effect on cytotoxicity demonstrated that the folate receptor/ligand-mediated cytotoxicity is exhibited by DTX-QDFA.

2.3 Folate receptor targeted liposomes for DTX

Folic acid (FA) is a common ligand which has been extensively investigated in drug delivery system. To better target the tumor tissues, DTX-loaded folate-conjugated PEG-liposomes were prepared and tested. The results indicated that the distribution of DTX in heart, brain and kidneys were decreased when administrated with folate-conjugated PEG-liposomes, whereas the accumulation of DTX in tumor tissues and its anti-tumor activity were significantly increased ($p < 0.05$) [31]. Li *et al.* [32] prepared folate-poly(PEG-cyanoacrylate-co-cholesteryl cyanoacrylate) (FA-PEG-PCHL)-modified liposomes for DTX loading. Compared with DTX solution and DTX-loaded liposomes, the DTX-loaded FA-PEG-PCHL-modified liposomes (FA-PDCT-L) demonstrated the strongest cytotoxicity against MCF-7 breast cancer cells and A-549 lung cancer cells, the greatest intracellular uptake especially in the nucleus, as well as the most powerful apoptotic efficacy. In pharmacokinetic studies, the area under the plasma concentration–time curve (AUC) of FA-PDCT-L was increased 3.82 and 6.23 times in comparison with that of the DTX-loaded liposomes and DTX solution, respectively. Meanwhile, a lower concentration of DTX was observed for FA-PDCT-L in liver and spleen, and a significantly higher concentration of DTX from FA-PDCT-L in tumors suggested that the presence of FA-PEG-PCHL in the liposomes resulted in greater accumulation of the drug in tumor tissue.

Muthu *et al.* [30] prepared folate receptor targeting TPGS-coated theranostic liposomes for DTX and QDs by the solvent injection method. Researchers used CLSM to visualize the targeting effect of prepared multifunctional liposomes (DTX-QDFA) in MCF-7 cells, and through the confocal images, it was easy to find that DTX-QDFA exhibited advantages compared with non-targeting liposomes (DTX-QD) for increasing cellular uptake by MCF-7 cells. After 24 h incubation in MCF-7 cells, the IC_{50} values were 0.23 ± 0.05 μg/ml which is greatly smaller than those for Taxotere (9.54 ± 0.76 μg/ml) and DTX-QD (1.56 ± 0.19 μg/ml). The IC_{50} value of DTX-QDFA with 1 mM free folate (1.10 ± 0.21 μg/ml) demonstrated that the folate receptor ligand-mediated cytotoxicity was exhibited by DTX-QDFA, but not free folate.

2.4 Transferrin receptor targeted liposomes for DTX

Transferrin receptor (TfR) is frequently overexpressed on epithelial cancer cells, therefore TfR-targeted liposomes is supposed to potentially improve tumor cell uptake, cytotoxicity and treatment efficacy of the encapsulated drug. Zhai *et al.* [33] prepared the DTX-loaded liposomes by polycarbonate membrane extrusion with the composition of hydrogenated soy phosphatidylcholine (HSPC)/egg phosphatidylcholine (PC)/cholesterol (Chol)/mPEG 2000-DSPE (HSPC/ePC/Chol/mPEG-DSPE), and try to make the liposomes obtain the property of TfR targeting by a post-insertion method. The cytotoxicity study showed that the cytotoxicity of TfR-targeted liposomes loading DTX was 3.6-fold greater than that of non-targeted control liposomes in KB cells, which meant that the receptor–ligand reaction mediated tumor-targeting indeed works.

The *in vitro* data of all the studies mentioned above look promising, however, it may not necessarily be effective *in vivo*. And also, *in vivo* metabolism, excretion, acute and chronic toxicity of DTX-loaded nanoformulations are needed for further investigation to prove that these liposomes are effective and safe.

3. Polymeric nanoparticles

As a sort of anticancer drug carrier, polymeric NPs have received an increasing attention for their ability to improve

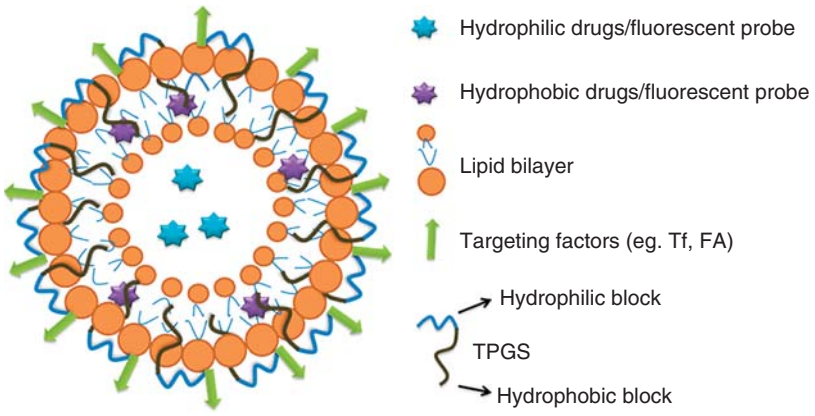

Figure 2. A simple example of a multifunctional liposome.

the efficacy of anticancer drugs [34,35]. It has been reported that natural and synthetic polymers enable NPs to maintain a prolonged circulation time in body, avoiding the elimination of reticuloendothelial system (RES) [36]. Additionally, the prolonged circulation time of polymeric NPs makes them accumulate into tumor tissue, thus, resulting in a disorganized and defective vascular architecture, which is referred to as EPR effect in tumor tissue [37].

The 'core-shell' structure is a major model of polymeric NPs. Biomimetic coating in the sterically stabilized nanocarriers enables them to maintain an increased longevity in circulation [14] and increase the feasibility to accumulate predominantly in pathological sites with compromised leaky vasculature. A biodegradable core-shell NP, which was assembled by amphiphilic block copolymers of poly(ε-caprolactone) (PCL) and poly(ethylene oxide) (PEO) with the weight ratio close to 2:1, was prepared for the passive targeting of DTX by a melting-sonication technique (MeSo) [38]. By using the MeSo, hydrated PEO segments with high chain mobility were located in the corona of NPs, and the PCL chains interacting with each other, formed a solid-like compact semi-crystalline core with strongly constrained motions. DTX entrapment in PEO-PCL NPs greatly decreased hemolysis of erythrocytes in comparison with a commercial DTX formulation, and the DTX-loaded NPs were more efficient in inhibiting the growth of breast and prostate cancer cells, and less toxic in experimental animal models in comparison with free DTX. Yuk *et al.* [39] prepared multicore Pluronic vesicle NPs for delivery of DTX (**Figure 3**). The Pluronic NPs composed of Pluronic F68 and PEG_{400}-containing DTX were stabilized by the phospholipid vesicle fusion. When DTX-loaded Pluronic NPs were mixed with phospholipid vesicles in the aqueous medium, DTX-loaded Pluronic NPs were incorporated into the phospholipid vesicles to form multicore vesicle NPs, which could significantly improve the stability of DTX-loaded Pluronic NPs and enable the NPs sustained release. Such release pattern led to the extended retention of DTX loading in multicore vesicle NPs in the blood stream at the tumor tissue. Antitumor efficacy and the time-dependent biodistribution verified that DTX-loaded multicore vesicle NPs could selectively target to tumor tissues, and were more effective in reducing the tumor volume compared with commercial DTX formulation. In addition, the empty NPs were also found to block tumor growth potentially by accumulation in the tumor tissue and changing the local environment.

In order to achieve more effective targeting, the surface of NPs was modified with peptides, nucleic acids, antibodies, aptamers or small molecules which could bind to antigens or receptors on the surface of tumor cells or malignant tissues. Gan and Feng [40] developed transferrin (Tf) conjugated NPs of poly(lactide)-D-α-tocopheryl polyethylene glycol succinate (PLA-TPGS) diblock copolymer for drug delivery across the blood–brain barrier (BBB). Kulkarni and Feng [41] prepared DTX-encapsulated poly(D,L-lactide-co-glycolide) NPs, and further modified the NPs with surfactants such as polysorbate-80 (Tween 80), F68 and poloxamer 407 (F127) to enhance cellular uptake of the NPs and increase the drug concentration in the brain tissues. Koopaei *et al.* [42] attached trastuzumab, a monoclonal antibody against human epidermal growth factor receptor 2 (HER2) antigens of cancer cells, to the maleimide groups on the surface of pegylated PLGA NPs as the targeting moiety for targeting delivery of DTX to human breast cancer cells. Sun and Feng [43] synthesized a novel system of DTX-loaded, trastuzumab-functionalized PLA-TPGS NPs for targeted and synergistic chemotherapy. And it was found that trastuzumab conjugated onto the PLA-TPGS NPs surface has two functions: one is to target HER2-overexpressing cancer cells and the other is to enhance the cytotoxicity of DTX through synergistic effects. Cho *et al.* [44] prepared DTX-loaded NPs composed of hyaluronic acid-ceramide (HA-CE) and Pluronic 85 (P85) for intravenous delivery of DTX. HA is an anionic, non-sulfated glycosaminoglycan distributed throughout connective, epithelial and neural tissues, and can bind to CD44 receptor which is overexpressed in various kinds of cancer cells [45,46]. In the study, the cellular uptake and the

Table 1. The encapsulation efficiency, *in vitro* and *in vivo* effects of liposomes.

Type of liposomes	EE%	*In vitro* effects		*In vivo* effects
		Cytotoxicity	Cellular uptake	
Targeting (DTX-QDFA) multifunctional TPGS-coated liposomes [30]	54.18 ± 0.62	After 24 h incubation, its IC_{50} value for MCF-7 cells was 0.23 ± 0.05 μg/ml, and it was 97.58% more efficient than Taxotere® after 24 h treatment	Folate decorated multifunctional liposomes (DTX-QDFA) exhibited advantages compared with non-targeting liposomes (DTX-QD) with regard to increased cellular uptake by MCF-7 cells	-
FA-PEG-PCHL liposomes (FA-PDCT4000-L) [32]	97.8 ± 1.6	In comparison with DTX solution, the IC_{50} for FA-PDCT4000-L decreased to 83.6, 71.1 and 58.4% in the MCF-7 line, and to 76.3, 65.3 and 66.3% in the A-549 line after 24, 48 and 72 h of treatment, respectively	The green fluorescent dots in cells incubated with coumarin-6-loaded FA-PEG4000-PCHL-modifed liposomes (FA-PCOU4000-L) were more concentrated than incubated with coumarin-6 and coumarin-6-loaded liposomes	In pharmacokinetic studies, the AUC of FA-PDCT-L was increased 3.82 and 6.23 times in comparison with the values for the DTX-loaded liposomes and DTX solution. Meanwhile, a lower concentration of DTX was observed for FA-PDCT-L in the liver and spleen, and a signifcantly higher concentration of FA-PDCT-L in tumors
Tf liposomes [33]	96.2 ± 2.5	In KB cells lines, Tf-targeted liposomal DTX had 3.6 times lower IC_{50} value (19.9 ± 4.2 nM) than that of non-targeted liposomal DTX and 1.7 times lower than that of DTX in Tween 80/ethanol	TfR-targeted liposomes showed much greater cellular uptake and liposome internalization than non-targeted control liposomes	-
DSPE-$mPEG_{2000}$-coated liposome [27]	55.56 ± 0.60	In C6 glioma cells, its IC_{50} value was 7.70 ± 0.22 μg/ml, and was 79.2% lower than that of Taxotere	The cellular uptake of the coumarin-6-loaded PEG-coated liposomes by C6 glioma cells was significantly higher than free coumarin-6	-

AUC: Area under the plasma concentration–time curve; DTX: Docetaxel; EE: Encapsulation efficiency; Tf: Transferrin.

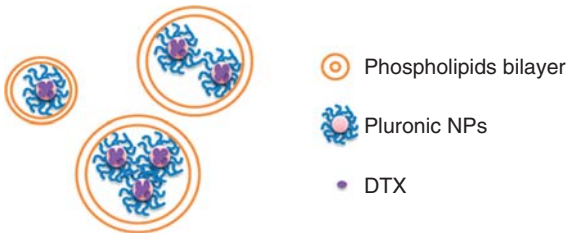

Figure 3. Structure of DTX-loaded multicore vesicle NPs.

in vivo tumor target ability studies demonstrated that the cellular uptake mechanism of the NPs was through receptor-mediated endocytosis, specifically a HA–CD44 receptor interaction, which appeared to be the principal driving force for tumor targeting. Additionally, incorporation of P85 in the HA-CE-based NPs resulted in the enhanced dissolution of DTX and MDR overcoming effect. Some important data (both *in vitro* and in *vivo*) in these researches mentioned above are presented in **Table 2**.

Although these polymeric NPs have had a good performance in *in vitro* experiments, there are fewer studies *in vivo* to prove their safety and efficacy. Moreover, these novel materials are newly synthesized, and there are not enough studies to prove their safety for human beings. Based on these problems, if a novel material is needed to be recognized, there are a lot of studies which have to be done.

4. Lipid nanoparticles

Lipid nanoparticles (LN) are basically including solid lipid nanoparticles (SLN) and nanostructure lipid carriers (NLC). LN are composed of lipids (pure lipids or a mixture of several lipid compounds such as triacylglycerols, fatty acids and oils) and surfactant (a single surfactant or co-surfactants). The SLN is generally a homogeneous matrix model that drugs could molecularly disperse in the lipid core or present in form of amorphous clusters, on the contrary, the NLC exists as a imperfect crystal model, and consists of a matrix with many voids and vacancies that are able to accommodate the drugs. In recent years, the lipid-based colloidal carriers receive particular attention, and their application in DTX delivery is widely developed.

It was reported that both SLN and NLC showed the controlled DTX-releasing and highly DTX-loading properties, but these characteristics were not much the same when different lipid compositions and the production methods were selected [47-49]. Also, SLN and NLC could significantly reduce the systemic toxicity (like allergenicity, vascular irritation and the long-term toxicities) of DTX in comparison with Taxotere and Duopafei, respectively [49,50]. The *in vitro* and *in vivo* effects of a topical example of DTX-loaded LN have been listed in **Table 3**, and in this table, the details of several other types of nanoformulations for DTX have also been listed. All the formulations included in **Table 3** are introduced and discussed in this review.

Drug targeting can be achieved by means of ligands placed onto the surface of LN. A DTX-loaded solid lipid nanoparticle (tSLN) targeted to hepatoma was designed and prepared with galactosylated dioleoylphosphatidyl ethanolamine modified as a ligand which could specifically target to ASGP receptor on hepatocellular carcinoma [51]. Hepatoma-targeted tSLNs were prepared by homogenization at elevated temperature, and the tSLNs showed a highly encapsulation efficiency (92.5 ± 3.7%) with a low burst effect at the first day and a sustained release for the next 29 days *in vitro*. The tSLNs showed the superior cytotoxicity against hepatocellular carcinoma cell line BEL7402 in comparison with Taxotere and non-targeted SLNs (nSLNs), but no detrimental effect on both healthy liver and liver with fibrosis. Moreover, the studies on biodistribution and cellular uptake demonstrated the tSLNs could increase accumulation of drug in tumor and promote cellular uptake by hepatoma cells, thereby enhance its antitumor effect.

Targeted DTX-loaded NLC was also investigated. For instance, VEGFR-mediated NLC was developed to target DTX to VEGFR-overexpressed tumor cells and tumor neovasculature endothelial cells, expecting to achieve the 'double targeting' (tumor- and vascular-targeting) effects [52]. Zhao *et al.* [53] synthesized a novel amphiphilic copolymer, folate-poly(PEG-cyanoacrylate-co-cholesteryl cyanoacrylate) (FA-PEG-PCHL) to modify DTX-loaded NLC, hoping such modification would lead to a long blood circulating and tumor targeting effect. After the intravenous administration of FA-DTX-NLC, the parameters of AUC_{0-t} and $AUC_{0-\infty}$ of DTX were significantly increased, with a decrease in clearance ($p < 0.01$), and thus, the mean retention time was prolonged ($p < 0.01$) in comparison with DTX injection and DTX-NLC. These results indicated that FA-PEG-PCHL-modified NLC could postpone the elimination of DTX and lead to a long circulating effect in blood. On one hand, the lipids matrix delayed DTX's degradation and slowed down the release of DTX from NLC, on the other hand, the hydrophilic part of PEG chains of the FA-PEG-PCHL swung on the surface of NPs ensuring efficient steric stabilization of FA-DTX-NLC in blood circulation. The tissue distribution study showed that the DTX concentrations in tumor and kidney for FA-DTX-NLC were significantly higher than those for DTX injection and DTX-NLC, and at 2 and 4 h, the drug levels of FA-DTX-NLC in tumor were significantly higher than those in other tissues. The targeting effect was folate-mediated, and folate receptor levels were overexpressed in tumor tissues and naturally rich in the kidney [54], this was reason that the FA-DTX-NLC could also target to kidney, but according to the data in the tissue distribution study, FA-DTX-NLC was more inclined to target to tumor.

5. Micelle

Micelles are spherical or globular structures with the diameters generally smaller than 100 nm. The polymeric

Table 2. The EE and DL, as well as *in vitro* and *in vivo* effects of polymeric NPs.

Type of polymeric NPs	EE%	DL%	*In vitro* effects		*In vivo* effects
			Cytotoxicity	Cellular uptake	
PEO-PCL NPs [38]	90 ± 5	8.7 ± 0.5	In DU145 cell lines, its IC_{50} value was < 0.195 ng/ml, while 1.36 ng/ml for free DTX; in MCF-7 cell lines, its IC_{50} value was < 0.04 ng/ml, and 0.35 ng/ml for free DTX	-	No severe side effects, sudden deaths or mean loss of more than 30% of the initial body weight were observed up to 28 days after administration with DTX-loaded NPs
PEO-PCL-PEO NPs [38]	91 ± 1	8.1 ± 0.2	The same as PEO-PCL NPs		The same as PEO-PCL NPs
PLA-TPGS/Tf NPs [40]	73.48 ± 2.28	-	In C6 glioma cells, the IC_{50} value of DTX-loaded PLA-TPGS/Tf NPs was 5.10 and 16.77 μg/ml for Taxotere®, which means that Tf-conjugated PLA-TPGS NPs formulation of DTX could be more efficient than Taxotere	In C6 glioma cells, the intracellular uptake of DTX-loaded formulations was in the order of Tf-conjugated PLA-TPGS NPs > PLA-TPGS NPs > PLGA NPs. In addition, the cellular uptake of the Tf-conjugated PLA-TPGS NPs was suppressed by excess free Tf	Reducing accumulation in the liver as well as in the spleen. The PLA-TPGS/Tf NPs formulation could deliver imaging/therapeutic agents across the BBB
Trastuzumab attached PEG-PLGA NPs [42]	-	-	DTX-loaded NPs were highly cytotoxic to BT-474 cells (HER2-positive), and more toxic than free DTX in the BT-474 cells	-	-
HA-CE/P85 NPs [44]	62.78 ± 0.04	7.72 ± 0.01	The viabilities (%) of U87-MG, MCF-7 and MCF-7/ADR cell lines exposed to blank NPs were over 80%, which indicated that blank NPs exhibited low cytotoxicity. The IC_{50} values of DTX-loaded NPs and Taxotere in the MCF-7/ADR cells (high CD44 expression and showing MDR to anti-cancer agents) were 200.94 and 966.43 ng/ml, respectively, which suggested that developed NPs could exert their function in the MDR-acquired tumor	Cellular uptakes were in order of MCF-7(high CD44 expression) > U87-MG (low CD44 expression), the difference may be explained by the HA–CD44 interaction	The results of *in vivo* tumor targeting efficiency in the MCF-7/ADR tumor-bearing mouse model showed that the fluorescence signal of the HA-CE-based NP with HA pre-injected animal group was lower than that of no pre-injection group for 24 h, the fluorescence intensity of the HA pre-injection group was only 43.9% that of the no pre-injection group

BBB: Blood–brain barrier; DL: Drug loading; EE: Encapsulation efficiency; NPs: Nanoparticles; Tf: Transferrin.

Table 3. The summary of *in vitro* and *in vivo* effects of different nanoformulations of DTX.

Type of nanoformulations	EE%	DL%	*In vitro* effects		*In vivo* effects
			Cytotoxicity	Cellular uptake	
DTX-loaded hepatoma-targeted tSLN [51]	92.5 ± 3.7	20.9 ± 1.0	The viability of BEL7402 cells treated with tSLNs did not demonstrate a significant difference compared with Taxotere® at low DTX concentrations (< 10 nM, $p > 0.05$), but the inhibiting activity of tSLNs increased with DTX concentration from 10 to 50 nM, and demonstrated a significant difference at 50 nM compared with the activity of Taxotere ($p < 0.01$)	Compared with nSLNs, tSLNs exhibited an increased accumulation of drug in tumor and more cellular uptake by hepatoma cells	tSLNs showed stronger tumor regression with a significant difference compared with Taxotere ($p < 0.01$) or nSLNs ($p < 0.05$) on day 21. And tSLNs had no detrimental effect on both healthy liver and liver with fibrosis
DTX-P80-PPI dendrimers [74]	67.6 ± 1.44	-	DTX exerted very potent cytotoxic effect and displayed IC_{50} value of 0.15 mM after incubation for a period of 24 h, which was significantly higher ($p < 0.001$) than DTX-PPI (0.9 mM) and DTX-P80-PPI (3.5 mM). After 48 h, extremely significant ($p < 0.001$) decrease in IC_{50} value of DTX-PPI and DTX-P80-PPI was observed, which was shifted from 0.9 – 0.25 mM and 3.5 – 0.3 mM for DTX-PPI and DTX-P80-PPI	In U87MG cell line, the cellular uptakes of different formulations were in the order of Tf-conjugated PPI dendrimers > P80-PPI dendrimers > 99mTcO4 – DTX > free 99mTcO4 (radioisotope)	DTX-P80-PPI reduced extremely significant tumor volume than DTX-PPI and free DTX ($p < 0.0001$), DTX-PPI had also shown very significant ($p < 0.001$) reduction in tumor volume as compared with control and free DTX; the median survival time for rats treated with DTX-loaded P80-anchored PPI dendrimers (42 days) was extended significantly as compared with DTXePPI (23 days, $p < 0.001$), free DTX (18 days, $p < 0.001$), receptor blocked group (15 days, $p < 0.001$) and control group (14 days, $p < 0.001$)
DLE [77]	96.9	-	The antitumor activities against human A549, BEL7402 and BCAP-37 cell lines *in vitro* showed there was no essential difference in the toxic effects between DLE and Taxotere	-	The pharmacokinetic study revealed when intravenous bolus dose was 5 mg/kg, the AUC of DLE was 3.29 times that of Taxotere, meanwhile, the clearance of DLE was 3.19 times lower than that of Taxotere in rats. In Beagle dogs, the AUC and clearance of DLE was 1.79 times higher and 2.02 times lower than that of Taxotere, respectively. Additionally, DLE did not change the distribution of DTX *in vivo*

AUC: Area under concentration–time curve; DAPI: 4′,6-diamidino-2-phenylindole; DL: Drug loading; DLE: DTX lipid emulsions; EE: Encapsulation efficiency; NC: Nanocapsule; nSLNs: Non-targeted SLNs; P-gp: P-glycoprotein; PPI: Poly(propyleneimine); SLN: Solid lipid nanoparticles; SN: Silica nanorattle; tSLN: targeted DTX-loaded solid lipid nanoparticle.

Table 3. The summary of *in vitro* and *in vivo* effects of different nanoformulations of DTX (continued).

Type of nanoformulations	EE%	DL%	*In vitro* effects		*In vivo* effects
			Cytotoxicity	Cellular uptake	
NCC/CTAB/DTX [83]	-	-	-	The nuclei of the KU-7 cells displayed pronounced staining with DAPI. There is clear evidence of cellular uptake of fluorescein as demonstrated by strong fluorescence emission from the cytoplasm of the cells. The uptake of fluorescein reached a maximum by 2 h with little increase in cytoplasmic fluorescence emission after longer incubations	-
Microparticles embedding DTX PLGA NCs [84]	-	4.34 ± 0.15	DTX NCs embedded in microparticles showed the same effect on the viability of WCR 256 as DTX solution did, and it increased cytotoxicity compared with the DTX solution	-	At a dose of 5 mg/kg, NCs in entero-coated microparticles by oral administration elicited a higher absolute bioavailability than both the DTX solution (276%) and the DTX NCs (400%) injected intravenously. The distribution study suggested that NCs released from the microparticles could penetrate the enterocytes, bypass P-gp, circumvent gut metabolism and accumulate within the lymphatic system, and drugs were progressively released into the circulation
SN-PEG-DTX (DTX-loaded PEGylated SN) [86]	-	32	SN-PEG-DTX showed a great lower IC_{50} for human liver cancer cell Hep-G2 after incubation for 72 h which was just only 7% of that of free DTX		SN-PEG-DTX did not have significant systematic toxicity in healthy ICR mice. Yet, the SN-PEG-DTX showed greater antitumor activity (about 15% higher) compared with Taxotere on the marine hepatocarcinoma 22 subcutaneous model

AUC: Area under concentration–time curve; DAPI: 4′,6-diamidino-2-phenylindole; DL: Drug loading; DLE: DTX lipid emulsions; EE: Encapsulation efficiency; NC: Nanocapsule; nSLNs: Non-targeted SLNs; P-gp: P-glycoprotein; PPI: Poly(propyleneimine); SLN: Solid lipid nanoparticles; SN: Silica nanorattle; tSLN: targeted DTX-loaded solid lipid nanoparticle.

molecules which can form micelles in a liquid environment generally have a hydrophobic end to form the central core of the sphere which serves as a microenvironment for loading of hydrophobic drugs (**Figure 4**). Meanwhile, the hydrophilic ends of the polymer molecules form a hydrophilic mantle to protect the inner structure and drugs in the liquid environment surrounding the micelles. The attractive advantages of micelles such as drug solubilization, controlled drug release, escaping from RES uptake and tumor targeting by EPR effect [55] make it a potential drug nanocarrier.

Many polymeric micelles have been developed for DTX loading, such as vitamin E TPGS micelles [56], Pluronic P123 polymeric micelles [57], mixed MPEG-PLA/Pluronic copolymer micelles [58], PEO-PCL micelles [59], DTX conjugated poly(ethylene glycol)-block-PCL (PEG-b-PCL-DTX) micelles [60] and monomethoxy-poly(ethylene glycol)-block-poly(L-lactide)/DTX (MPEG-b-PLLA/DTX) conjugates [61] as shown in **Table 2**, all these polymeric micelles could more or less exhibit the quality of solubilization, protecting the anticancer drug DTX from degradation, enhancing bioavailability of DTX, overcoming some of the limitations on its MDR in cancer therapy, and improving the tumor therapeutic effects of DTX in the chosen organs.

5.1 Receptor-mediated targeting of polymeric micelles

Micelles with more specific targeting effect have been explored in various ways. Zhang *et al.* [62] used somatostatin analog octreotide (OCT), which regulates potent inhibition of hormone and growth factor secretion, as the targeting bullet conjugated on the surface of micelles to achieve the enhanced intracellular delivery of DTX and superior antitumor efficacy toward specific malignant tumor model. The OCT-modified DTX-loaded PEG-b-PLA polymeric micelles were prepared by film dispersion method, the optimal modification ratio of OCT on micelle surface was 5% and DTX encapsulation efficiency was 87%. Flow cytometry and confocal microscopy studies showed that the internalized DiI fluorescence intensity of fluorescent probe DiI-loaded micelles with OCT modification (OCT-PM-DiI) had 1.5-, 5.1-, 5.1-folds that of DiI-loaded micelles without OCT modification (PN-DiI) after 1, 3 and 6 h incubation at 37°C in NCI-H446 cells, respectively, such enhanced intracellular delivery efficiency of OCT-PM-DiI was attributed to somatostatin receptor-mediated endocytosis in NCI-H446 cells.

FA as a ligand is also used in micelle drug delivery system for tumor targeting. Mi *et al.* [63] developed a micelle system with a newly synthesized $TPGS_{2k}$ polymer, which showed lower CMC of 0.0219 mg/ml compared with 0.2 mg/ml for traditional TPGS micelles, to achieve sustained and controlled delivery of DTX. Furthermore, FA was conjugated to the $TPGS_{2k}$ micelles for targeted drug delivery. From the *in vitro* DTX release profile, an initial burst of 16.93% for the $TPGS_{2k}$ micelles and 19.78% for the FA-conjugated $TPGS_{2k}$ micelles were observed in the first 12 h. Such initial burst caused by those DTX located near the outer surface of the micelles was considered to be useful to inhibit the growth of tumor in the beginning of the treatment. In the following hours, the cumulative release sustainably increased, which ensured the micelles to possess the sustained property. The IC_{50} value, an indicator of the targeting effects for the FA-conjugated $TPGS_{2k}$ micelles, was 0.1780, 0.1520 and 0.1140 mg/ml for MCF-7 cancer cells after 24, 48 and 72 h for the FA-conjugated micelles, which was 99.8, 88.1 and 23.0% lower than that for commercial Taxotere and 66.2, 39.4 and 51.1% lower than that for $TPGS_{2k}$ micelle formulation, respectively. In addition, as a kind of 'mitocans', $TPGS_{2k}$ micelles without drug showed certain cytotoxicity at first 24 and 48 h, which demonstrated the possible synergistic effects of $TPGS_{2k}$ with DTX.

5.2 Thermosensitive polymeric micelles

With the development and application of large-scale hyperthermia instruments, the thermal targeting has become much easier to implement and control [64]. Thermosensitive drug carrier which relies on local heating to control the release of drugs undergoes a structural transition as a response of temperature increase, which is helpful for the deposition of the drug and easier drug absorption by cells. Hyperthermia also increases the permeability of tumor vasculature preferentially when compared with that of normal vasculature, facilitating the delivery of drugs to tumors [65]. Additionally, hyperthermia has a particular advantage of synergetic effects to kill malignant tumor cells when combined with chemotherapies [66].

Aiming at evaluating the novel DTX-loaded micelle based on the biodegradable thermosensitive copolymer poly(*N*-isopropylacryl-amide-co-acrylamide)-b-poly(DL-lactide) whose low critical solution temperature (LCST) was 41°C. Liu *et al.* [67] investigated the cytotoxicity of DTX-loaded micelles in three different tumor cell lines with standard MTT assays, and found that IC_{50} values of cell lines BGC823 treated with the thermosensitive DTX-loaded micelles and conventional DTX formulation were 238.2 and 190.9 μg/ml, respectively, and the results were very similar for the cell lines SMMC-7721 and LLC, which meant that the *in vitro* cytotoxicity of the thermosensitive DTX-loaded micelles was lower than that of the conventional DTX formulation at 37°C. However, hyperthermia greatly enhanced the efficacy of the thermosensitive drug-loaded micelles, for instance, the IC_{50} of cell lines BGC823 treated with the thermosensitive DTX-loaded micelles with the help of hyperthermia was five times lower than that of conventional DTX formulation under the same conditions. Moreover, the tumor inhibition rate of the thermosensitive DTX-loaded micelles with the help of hyperthermia was higher than that of conventional DTX formulation (82.1 vs 53.0%, $p < 0.05$) and DTX-loaded micelles without the help of hyperthermia (82.1 vs 41.2%, $p < 0.05$). This study indicated that the thermosensitive micelles are worthy to develop in clinic.

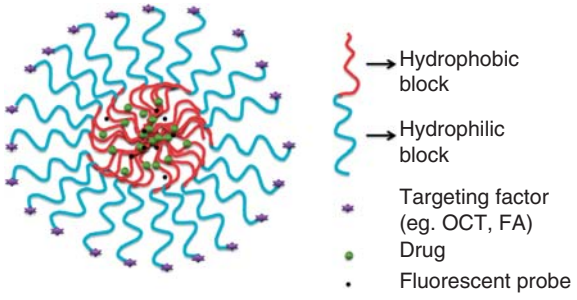

Figure 4. Structure of diblock polymer micelles with multi-function.

5.3 pH-sensitive polymeric micelles

Polymeric micelles made from pH-sensitive block copolymers have been designed for targeting tumor acidity or endosomal pH in tumor cells. They possess certain segments that have physical or chemical properties responding to small changes in environmental pH, which enables them to adjust the biodistribution of the micelles and the interactions with tissues and cells. Such properties are helpful to overcome the problems associated with free chemo-agents, including lack of tumor selectivity, non-specific toxicity and the development of MDR in various tumor cells [68].

Poly(2-ethyl-2-oxazoline)-poly(D,L-lactide) (PEOz-PDLLA), a kind of pH-sensitive amphiphilic block copolymer whose CMC was 1.0 mg/l, was synthesized and used to prepare DTX-loaded pH-sensitive block copolymer micelles by film dispersion method [69]. The drug-loaded amount and entrapment efficiency of the micelles was 15.0 and 91.1%, respectively. The *in vitro* release behavior of DTX from polymeric micelles was investigated with dialysis method. It was found that in pH 7.4 PBS (phosphate buffered saline), DTX from the micelles was released in a sustained manner, while in PBS at pH 5.0, DTX was released more rapidly. The results showed that this nanoformulation for DTX was sensitive to the small changes of surrounding pH from 7.4 (normal physiological environment) to 5.0 (endosomal pH in tumor cells).

6. Dendrimers

Dendrimers are three-dimensional architecture in which monodispersed macromolecules exist as regular tree-like branches. The branch lengths are sterically limited and each single dendrimer is sphere shaped with small molecular size (1 – 100 nm) but high molecular weights (**Figure 5**). The drug delivery capability of dendrimer was attributed to the properties including multivalency, monodisperse, a large number of peripheral end groups, host–guest entrapment and interior cavities [70]. Active molecules could be either shielded in the dendritic channels in the interior structure or attached to functional groups on the dendrimer surface [71,72]. Dendrimers can host both hydrophobic and hydrophilic molecules, and are useful nanocarriers for genes, drugs and anticancer agents. There is a report on a positive preclinical trial results of dendrimer–DTX carried out by SPL (Sylvania Platinum Ltd.). According to this report, SPL's dendrimer–DTX was more effective in treating breast cancer in animals than Taxotere and had a longer duration of effect than Taxotere. Moreover, its solubility in water was greater than Taxotere, which was meaningful for avoiding the high doses of cortisone and the need for inclusion of formulation components thought to cause the severe allergic reactions and fluid retention. And next, SPL is approved to perform a PhaseI/II clinical trial to prove the safety and equivalency compared with Taxotere.

Dendrimers can be anchored with ligands which interact with receptors overexpressed on tumors and might elicit receptor-mediated targeting. Based on reports that apolipoproteins (e.g., ApoE) were believed to be adsorbed on polysorbate 80 (P80)-anchored nanocarriers, which led to the interaction between the nanocarriers and LDL receptors on BBB and subsequent endocytosis [73]. Gajbhiye *et al.* [74] prepared P80-anchored poly(propyleneimine) (PPI) dendritic nanoconjugate (P80-PPI). P80-PPI dendritic nanoconjugate (10 mM) and DTX (100 mM) were taken in PBS (pH 7.4), and the mixture was magnetically stirred (50 rpm) for 48 h using teflon beads. After evaporation and dialysis, the DTX-loaded P80-PPI dendrimer was lyophilized and used for further evaluation. The DTX entrapment of P80-PPI dendrimers was 67.61 ± 1.44%. Gamma scintigraphy and biodistribution studies confirmed the targeting efficiency and higher brain accumulation of P80-PPI dendrimer into the brain, and the *in vivo* anti-cancer activity in brain tumor bearing rats further showed that DTX-loaded P80-PPI dendrimers reduced the tumor volume (by more than 50%) extremely significantly ($p < 0.0001$).

7. Nanoemulsion

Nanoemulsions, usually spherical, exist as water-in-oil or oil-in-water form with high stability, so the core of the particle is either water or oil, which enables it to possess unique property of acting as super-solvent for both hydrophobic and hydrophilic molecular. Li *et al.* [75] prepared an oil-in-water nanoemulsion by high-pressure homogenization method for DTX loading which was composed of medium-chain triglyceride, oleic acid, egg lecithin and poloxamer, and such DTX-loaded nanoemulsions were well stable even under high centrifugation owe to its high surface charge with the zeta potential of -33.9 mV.

Lipid nanoemulsions, formed mainly by soya bean oil and phospholipids, have been reported to possess versatile advantages like high drug loading capacity, stability during long-term storage, reduced irritation or toxicity of the incorporated drugs, a reduction in drug hydrolysis and no precipitation during administration [76]. Based on these reports, Zhao *et al.* [77] selected 2.5% (w/v) soybean oil, 7.5% (w/v) MCT, 3.0% (w/v) egg lecithin, 0.025% (w/v) oleic

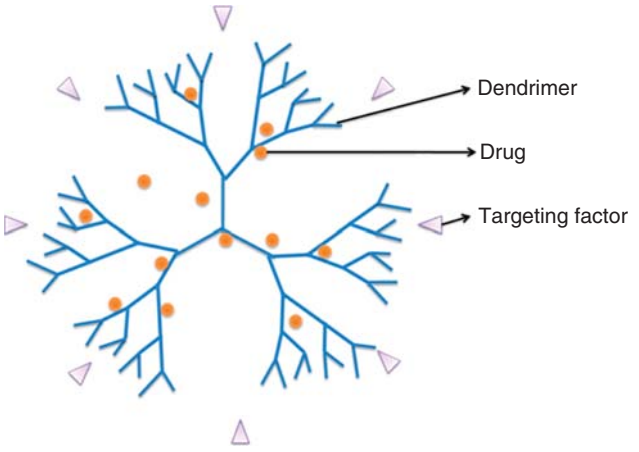

Figure 5. Structure of drug-loaded and active targeted dendrimer.

acid and 0.08% (w/v) DTX as the oil phase, and chose 0.2% (w/v) F68, 0.02% (w/v) sodium bisulfite and 2.5% (w/v) glycerin as the aqueous phase, and prepared the intravenous DTX lipid emulsions (DLE) by high-speed shear mixing and high-pressure homogenization. The mean particle size, the zeta potential and the pH value of the DLE was 144.4 ± 41.90 nm, -29.36 mV and 5.34, respectively, and the entrapment efficiency of DTX in DLE was 96.9%. The pharmacokinetic study revealed when intravenous bolus dose was 5 mg/kg, the AUC of DLE was 3.29 times that of Taxotere, meanwhile, the clearance of DLE was 3.19 times lower than that of Taxotere in rats, and as the dose increased (10, 20 mg/kg), the trend was essentially the same. In Beagle dogs, the AUC and clearance of DLE was 1.79 times higher and 2.02 times lower than that of Taxotere, respectively. The tissue distribution study showed that the distribution profiles of DLE and Taxotere were similar, DLE did not change the distribution of DTX *in vivo*. Moreover, the antitumor activities against human A549, BEL7402 and BCAP-37 cell lines *in vitro* showed there was no essential difference in the toxic effects of DLE and Taxotere. However, the long-term toxic study demonstrated the DLE was less toxic than Taxotere, and such toxic effects could be reversed. However, there is still much work to be done (e.g., *in vitro* cytotoxicity and *in vivo* antitumor activity) to help us to have a comprehensive understanding of this nanoformulation.

8. Other nanoformulation

8.1 Nanocrystalline cellulose

Cellulose has been wildly investigated as a pharmaceutical material which has excellent compaction properties when blended with other excipients [78-80]. In recent years, nanocrystalline cellulose (NCC) is received rising attention. NCC, a formation of nanodimensional cellulose rods, is extracted from lignocellulosics by acidic extraction methods. NCC possesses qualities of high aspect ratio, large surface area and tremendous stiffness and strength, which makes NCC to be used as a nanocomposite material [81,82].

Based on the fact that there are abundant surface hydroxyl groups on NCC, Jackson *et al.* [83] modified the surface of NCC with the cationic surfactant, cetyltrimethylammonium bromide (CTAB) for the binding and release of hydrophobic drugs (including DTX). NCC/CTAB nanocomplexes could significantly bind quantities of the non-ionized hydrophobic anticancer agents, and it was found that increased amounts of CTAB resulted in increased drug binding, at the highest CTAB concentration (12.9 mM), the binding efficiency of DTX and paclitaxel to the NCC/CTAB nanocomplexes was approximately 90 – 100%, and these drugs were released in PBS in a controlled manner over several days. At last, the cell binding and uptake studies demonstrated that the NCC/CTAB nanocomplexes could bind to KU-7 bladder cancer cells and efficiently deliver hydrophobic fluorescent probe to the cytoplasm of these cells.

8.2 Nanocapsule

Nanocapsule (NC) is a kind of vesicular system with a core or central cavity for drugs' capsulizing. Its inner core could be solid, liquid or gas with an aqueous or oily environment. And its outer shell of polymeric membrane could bind different targeting ligands or antibodies which attach NC to some certain tissues and cells.

Nassar *et al.* [84] prepared DTX-loaded PLGA NCs by spray-drying technique, and embedded the drug-loaded particles in entero-coated microparticles to find if the nanotransporter formulation could bring a better bioavailability and anticancer effect. The pharmacokinetic study showed that at a dose of 5 mg/kg, NCs in entero-coated microparticles by oral administration elicited a higher absolute bioavailability than both the DTX solution (276%) and the DTX NCs (400%) injected intravenously. When at a oral dose of 10 mg/kg, such NCs in entero-coated microparticles showed much higher C_{max} (2.299.6 ± 615.5 ng/ml for batch I and 2.986.9.5 ± 1.859.4 ng/ml for batch II) than DTX solution combined with blank NCs embedded in microparticles (132.5 ± 120.4 ng/ml). The distribution study suggested that NCs released from the microparticles could penetrate the enterocytes, bypass P-gp, circumvent gut metabolism and accumulate within the lymphatic system, and drugs were progressively released into the circulation. In the blood circulation, DTX was either in free form or bound to albumin without encapsulated. *In vitro* experiments demonstrated that DTX NCs embedded in microparticles showed the same effect on the viability of WCR 256 as DTX solution did, and it increased cytotoxicity compared with the DTX solution. Such results suggested that the activity of DTX was not altered when encapsulated in the nanotransporter formulation.

8.3 Silica nanorattle

Mesoporous silica is a promising nanomaterial for biomedical applications; especially for cancer therapy, there have been some studies reported in the past decade. Li *et al.* [85] synthesized silica nanorattles (SNs) by selective-etching method as a drug delivery system. It had great advantages, such as high drug loading amount, surface functionalization and bioconjugation with biomolecules, controllable *in vivo* biodistribution and suitable for large-scale synthesis. In their study [86], DTX-loaded PEGylated silica nanorattle (SN-PEG) was synthesized. In the *in vitro* experiments, SN-encapsulating DTX showed a great lower IC_{50} for human liver cancer cell HepG2 after incubation for 72 h which was just only 7% of that of free DTX. The *in vivo* toxicity assessment showed that the SNs and SN-PEG-DTX did not have significant systematic toxicity in healthy ICR mice. Yet, the SN-PEG-DTX showed greater antitumor activity (about 15% higher) compared with Taxotere on the marine hepatocarcinoma 22 subcutaneous model. All the studies demonstrated that SN-PEG is a promising candidate for DTX delivery with low toxicity and high antitumor efficacy.

9. Expert opinion

Overall, nanotechnology has great potential for DTX delivery because of their advantages as compared with other mode of drug delivery such as small size, drug solubilization, controlled drug release, escaping from RES uptake and tumor targeting by EPR effect. Though several drug delivery NPs approved by FDA are available for cancer treatment, there is none for DTX delivery. To fully explore these potentials, more trials are needed to evaluate the anticancer efficacy and toxicity of nanoformulated DTX in both small and large animal models, even in patients with cancer in Phase I/II clinical trials.

Although general nanoformulations have the property of passive targeting, their tissue specificities are not as ideal as expected, which means that they can spread to healthy tissues when delivering the drug to the pathologic organism. This is a serious problem because the toxicity of drugs and pharmaceutical excipients may severely damage normal tissues and cells. To address these problems, intense investigations on novel strategies (e.g., antibody- or peptide-targeted delivery) for active targeting are being carried out, expecting to enhance the anticancer efficiency and at the same time decrease the toxicity to normal tissues. However, the development of active targeting is accompanied by other hurdles. One of which is that synthesis of materials with ligands conjugated is often complex and expensive. Therefore, many of these materials are not feasible for large-scale production at the present time. Another problem is that these novel materials are newly synthesized, and have not been thoroughly tested for their safety in patients. Facing these problems, when a novel material is identified and need to be synthesized in large scale, a great deal of studies must be completed and its safety should be established.

Another issue that needs to be addressed is that most of the current studies on DTX-loaded nanoformulations focus on the *in vitro* and *in vivo* antitumor activities and biodistribution, less investigations have been reported on the *in vivo* metabolism, excretion, acute and chronic toxicity as well as *in vitro* physicochemical stability of nanoformulations loading DTX. Research in these areas must be conducted to make these nanoformulations for DTX applicable in clinic and commercially available.

Despite the daunting challenges associated with the current DTX-loaded nanoformulations, nanotechnology is the mainstream method and holds great potential in its applications. In the foreseeable future, nano-sized DTX-loaded delivery systems for cancer therapy will continue to develop and expand.

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Declaration of interest

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- **The SN as a novel strategy for DTX loading and delivery was investigated and nicely discussed in this paper.**

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