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Paclitaxel Nano-Delivery Systems: A Comprehensive Review

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Abstract

Paclitaxel is one of the most effective chemotherapeutic drugs ever developed and is active against a broad range of cancers, such as lung, ovarian, and breast cancers. Due to its low water solubility, paclitaxel is formulated in a mixture of Cremophor EL and dehydrated ethanol (50:50, v/v) a combination known as Taxol. However, Taxol has some severe side effects related to Cremophor EL and ethanol. Therefore, there is an urgent need for the development of alternative Taxol formulations. The encapsulation of paclitaxel in biodegradable and non-toxic nano-delivery systems can protect the drug from degradation during circulation and in-turn protect the body from toxic side effects of the drug thereby lowering its toxicity, increasing its circulation half-life, exhibiting improved pharmacokinetic profiles, and demonstrating better patient compliance. Also, nanoparticle-based delivery systems can take advantage of the enhanced permeability and retention (EPR) effect for passive tumor targeting, therefore, they are promising carriers to improve the therapeutic index and decrease the side effects of paclitaxel. To date, paclitaxel albumin-bound nanoparticles (Abraxane®) have been approved by the FDA for the treatment of metastatic breast cancer and non-small cell lung cancer (NSCLC). In addition, there are a number of novel paclitaxel nanoparticle formulations in clinical trials. In this comprehensive review, several types of developed paclitaxel nano-delivery systems will be covered and discussed, such as polymeric nanoparticles, lipid-based formulations, polymer conjugates, inorganic nanoparticles, carbon nanotubes, nanocrystals, and cyclodextrin nanoparticles.

Abbreviations: Ab: Antibody; Au NPs: Gold Nanoparticles; AUC: Area Under the Curve; BBB: Blood-Brain Barrier; BrC16: 2'-2-Bro-Mo-hexadecanoyl; Brij 78: Polyoxyl 20-Stearyl Ether; BSA: Bovine Serum Albumin; C22-PX: 2'-Behenoyl-Paclitaxel Conjugate; CD: Cyclodextrin; CHO: Cholesterol; C_{max}: Maximum Concentration; CMC: Critical Micelle Concentration; CNT: Carbon Nanotubes; DHA: Docosahexaenoic Acid; DLPC: 1,2-Dilauroylphosphatidylcholine; DMAB: Didodecyldimethylammonium Bromide; DNA: Deoxyribonucleic Acid; DOPC: 1,2-Dioleoyl-Sn-Glycero-3-Phosphocholine; DOTAP: N-[1-[2,3-Dioleoyloxy]Propyl]-N,N,N-Trimethyl-Ammonium Methylsulfate; DPPC: Dipalmitoyl-Phosphatidylcholine; DSPC: 1,2-Distearoyl-Sn-Glycero-3-Phosphocholine; EE: Entrapment Efficiency; EPC: Egg Phosphatidylcholine; EPR: Enhanced Permeability and Retention; FA: Fatty Acid; FITC: Fluorescein Isothiocyanate; h: Hour; HA: Hyaluronic Acid; HER2: Human Epidermal Growth Factor Receptor 2; HO-GC: Hydrotropic Oligomer-Glycol Chitosan; HPG: Hyperbranched Polyglycerol; HPMA: N-[2-Hydroxypropyl]Methacrylamide; HSA: Human Serum Albumin; HSPC: Hydrogenated Soybean Phosphatidylcholine; IC_{50}: Half Maximal Inhibitory Concentration; i.p: Intraperitoneal; i.v: Intravenous; kg: Kilogram; LRP1: Low-Density Lipoprotein Receptor-Related Protein 1; mAb: Monoclonal Antibody; MDR: Multiple Drug Resistance; mg: Milligram; min: Minute; mL: Milliliter; MMT: Montmorillonite; MNP: Magnetic Nanoparticle; mPEG: Methoxy Poly[Ethylene Glycol]; MTD: Maximum Tolerated Dose; NC: Nanocapsule; NSCLC: Non-Small Cell Lung Cancer; ng: Nanogram; NMR: Nuclear Magnetic Resonance; NP: Nanoparticle; OSA: Octyl-Modified Bovine Serum Albumin; PACA: Poly[Alkyl Cyanoacrylate]; PAMAM: Poly[Amidoamine]; PbAE: Poly[β-Amino Ester]; PBCA: Poly[Butyl Cyanoacrylate]; PCL: Poly [ε-Caprolactone]; PE: Phosphatidyl Ethanolamine; PEEP: Poly[Ethyl Ethylene Phosphate]; PEG: Poly[Ethylene Glycol]; PEG-DSPE: Polyethylene Glycol-Distearoylphosphatidylethanolamine; PEI: Polyethylenimine; PEO-b-PCL: Poly[Ethylene Oxide]-block-Poly[ε-Caprolactone]; PEO-PbAE: Poly[Ethylene Oxide]-Modified Poly[β-Amino Ester]; PEO-PPO-PEO: Poly[Ethylene Oxide]-Poly[Propylene Oxide]-Poly[Ethylene Oxide]; PEtOz: Poly[2-Ethyl-2-Oxazoline]; PG: Poly[L-Glutamic Acid]; PGG: Poly[L-γ-Glutamyl-Glutamine]; P-gp: P-glycoprotein; PLA: Poly[L-Lactide]; PLGA: Poly[Lactic-Co-Glycolic Acid]; Pluronic P85: Poly[Oxyethylene-b-Oxypropylene-b-Oxyethylene]; PPEEA : Poly[2-Aminoethyl Ethylene Phosphate]; PVA: Poly[Vinyl Alcohol]; PX: Paclitaxel; Ref: Reference; RES: Reticuloendothelial System; RHAMM: Hyaluronan-Mediated Motility Receptor; RNA: Ribonucleic Acid; s.c.: Subcutaneous; SD: Standard Deviation; SEC: Size Exclusion Chromatography; siRNA: Small Interfering RNA; SLN: Solid Lipid Nanoparticle; SPAnNa: Poly [Aniline-co-Sodium N-[1-One-Butyric Acid] Aniline]; SSMM: Sterically Stabilized Mixed Micelle; $t_{1/2}$: Half-Life; TEM: Transmission Electron Microscopy; TPGS: D-α-Tocopheryl Polyethylene Glycol 1000 Succinate; μg: Microgram; μL: Microliter; VIP : Vasoactive Intestinal Peptide; vs: Versus

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Paclitaxel and its Limitations

Paclitaxel (PX), isolated from the bark of Pacific Yew (*Taxus brevifolia*), which was first discovered by Mrs. Monroe E. Wall and Mansukh C. Wani, is a white crystalline powder with the melting point

of ~210°C (Figure 1). It is one of the most effective chemotherapeutic drugs and is mainly used to treat lung, ovarian, and breast cancer, etc [1]. The mechanism of action of PX is to promote and stabilize microtubules and inhibit late G2 or M phases of cell cycle, thereby causing the cell death. The major limitation of PX is its low water solubility (~0.4 μg/mL); thus, it is formulated in organic solvents of polyoxyethylated castor oil (Cremophor EL) and dehydrated ethanol (50/50, v/v) under the trademark "Taxol". However, Cremophor EL is known to cause serious side effects, such as hypersensitivity reactions [2]. As a result, prolonged infusion time and pretreatments are required. Moreover, the presence of Cremophor EL alters the pharmacokinetic profile of PX *in vivo* which was described as unpredictable non-linear plasma pharmacokinetics when PX was formulated in Cremophor EL [3]. In addition, PX is a substrate of P-glycoprotein (P-gp), which actively pumps PX out of the cells and induces drug resistance [4]. To overcome this problem, several P-gp inhibitors, such as verapamil [5] and PSC 833 [6], were co-administered with Taxol but the results were disappointing due to their toxicity and/or alteration of PX pharmacokinetics and biodistribution. Nano-delivery systems are promising vehicles in drug delivery because they improve solubility of hydrophobic drugs, such as PX, and generally have low toxicity as well. Abraxane®, a PX albumin-bound NP formulation with the particle size of ~130 nm, was approved by the FDA in 2005 for the treatment of metastatic breast cancer. This formulation had demonstrated some advantages in terms of reduced toxicity compared to Taxol. In addition, the total dose can be administered within 30 min without pretreatment. However, whether Abraxane® could improve survival and address P-gp-mediated drug resistance is still unclear. Therefore, the alternative PX formulations are still in demand. In this review, various nanoparticle (NP) systems for the delivery of PX will be addressed, such as polymeric NPs, lipid-based NP formulations, polymer conjugates, inorganic NPs, carbon nanotubes, nanocrystals, cyclodextrin NPs, etc.

Advantages of Nanoparticle-Based Paclitaxel Delivery Systems

Nanoparticle delivery systems have attracted increasing attention in recent years, especially for cancer therapies. As an effective chemotherapeutic agent, PX has been formulated in various nano-delivery systems which have several advantages over the standard-of-care therapy. First, the aqueous solubility of PX can be greatly enhanced when it is conjugated with water-soluble polymers, or encapsulated into lipid-based NPs. Second, they are small in size (several to several hundred nanometers in diameter), which enables the preferential delivery of PX into the tumor site due to the enhanced permeability and retention (EPR) effect. Third, they can escape the recognition of reticuloendothelial system (RES) in healthy tissues and therefore reduce the side effects of the drug. As a consequence, higher maximum
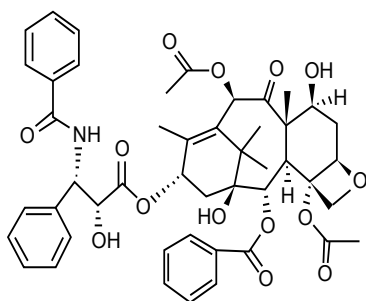
tolerated doses (MTD) of NPs are realized. It should be noted that, in general, the addition of polyethylene glycol (PEG) on the surface of NPs is required to avoid RES clearance [7]. Fourth, the pharmacokinetic profiles of the drug from NPs is improved, for example, increasing the half-life and tumor accumulation of PX. Last, but not the least, the surface of PX NP systems can be functionalized with active ligands for targeting purpose, which in-turn will further increase the tumor uptake and decrease the side effects of the drug. For more details about the advantages of NP-based drug delivery systems, please refer to the referenced review articles [8-12].

Polymeric Nanoparticles

A summary of PX-loaded polymeric NPs is shown in Table 1.

Poly (lactic-co-glycolic acid) (PLGA) Nanoparticles

PLGA is one of the most widely used biodegradable co-polymers for the development of nano-delivery systems because it undergoes hydrolysis in the body and produces non-toxic products of lactic acid and glycolic acid, and eventually carbon dioxide and water. Since the body effectively deals with both degradants, the systemic toxicity associated with PLGA is minimal.

PX-loaded PLGA NPs have been engineered by different methods, such as o/w emulsion-solvent evaporation [13,14], nanoprecipitation [15] and interfacial deposition methods [16]. In most cases, PX was released from PLGA NPs in a biphasic pattern with a fast initial release during the first 1-3 days followed by a slow and continuous release [13,14,16-18]. PX-encapsulated PLGA NPs demonstrated enhanced *in vitro* cytotoxicity as compared to free PX in various cancer cell lines, such as glioma C6 cells [17], NCI-H69 human small cell lung cancer cells [16], MCF-7 [18] and HeLa cells [15,18]. Furthermore, *in vivo* PX-loaded PLGA NPs showed significantly better tumor growth inhibition effect with transplantable liver tumors [15].

The surface of PLGA NPs was modified for improved drug delivery. Chitosan-coated PLGA NPs exhibited slower *in vitro* drug release compared to non-coated PLGA NPs and significantly changed the zeta potential from the negative charge of -30.1 mV for PLGA NPs alone to the positive charge of 26 mV, which facilitated drug cell uptake than uncoated NPs [19]. Chakravarthi et al. [20] showed a 4-10-fold increase in cellular association of PX and enhanced cytotoxicity when applied chitosan-modified PLGA NPs. In addition to chitosan, didodecyldimethylammonium bromide (DMAB), a cationic surfactant, was also applied to absorb on the surface of PX-loaded NPs by electrostatic attraction. Upon the addition of DMAB, the negatively-charged NPs shifted to become positively-charged [21]. This DMAB modified PX-incorporated PLGA NPs completely inhibited intimal proliferation in a rabbit vascular injury model [22].

PLGA NPs were also optimized using different emulsifiers. It is known that the employed emulsifiers/stabilizers could have strong influence on the properties of produced NPs, such as morphology, particle size, drug entrapment efficiency, *in vitro* release behavior, cellular uptake, *in vitro* cytotoxicity, pharmacokinetics and biodistribution, and as a consequence therapeutic efficacy [23]. Poly (vinyl alcohol) (PVA) is the most commonly used emulsifier. Other emulsifiers were also applied in PLGA NPs. For example, when d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) was utilized in PX-loaded PLGA NPs as the surfactant emulsifier, the PLGA/TPGS NPs could achieve drug encapsulation efficiency of 100% [24], better controlled drug release kinetics [25], and enhanced cellular uptake and cytotoxicity [26] compared to that of PVA-emulsified PLGA NPs. The TPGS-emulsified

Figure 1: Chemical Structure of PX.

Polymer	Modification	NP Preparation Method	% EE*	Status	References
PLGA	—	emulsion-solvent evaporation	85	*in-vitro*	[14]
	PLGA, PLGA-PEG, PCL-PEG	nanoprecipitation	70	*in-vivo*	[15]
	Poloxamer 188	interfacial deposition	>90	*in-vitro*	[16]
	TPGS (emulsifier)	emulsion-solvent evaporation	100	*in-vitro*	[24]
	DLPC (emulsifier)	emulsion-solvent evaporation	15-56	*in-vitro*	[28]
	DPPC (emulsifier)	emulsion-solvent evaporation	34-45	*in-vitro*	[29]
	chitosan	emulsion-solvent evaporation	75-79	*in-vitro*	[19]
	DMAB	emulsion-solvent evaporation	47	*in-vivo*	[21]
	MMT	emulsion-solvent evaporation	~50	*in-vitro*	[30]
	MMT, HER2 (targeting)	emulsion-solvent evaporation	~50	*in-vitro*	[31]
	RGD (targeting)	emulsion-solvent evaporation	60-65	*in-vivo*	[32]
	Pluronic P85, transferrin (targeting)	nanoprecipitation	70-76	*in-vivo*	[33]
PCL	PEO-PCL	solvent displacement	>95	*in-vivo*	[63,64]
	PCL-pluronic F68	emulsion-solvent evaporation	84	*in-vivo*	[72]
	PCL-pluronic F68, DMAB	modified solvent displacement	76-88	*in-vivo*	[193]
	mPEG-PCL, Angiopep (targeting)	emulsion and evaporation	90	*in-vivo*	[67,68]
	mPEG-PCL	solid dispersion	98	*in-vivo*	[69]
	PVP-b-PCL	modified nanoprecipitation	85	*in-vivo*	[73]
	PEG-PCL	co-solvent extraction	—	*in-vivo*	[66]
	PEG-PCL, folic acid (targeting)	dialysis	—	*in-vitro*	[70]
	PCL-g-PVA	dialysis	—	*in-vitro*	[74]
	PEtOz-PCL	dialysis	5-76	*in-vitro*	[75]
	PCL-PEEP, galactosamine, (targeting)	dialysis	—	*in-vitro*	[76]
	mPEG-PCL-PPEEA	emulsion-solvent evaporation	>90	*in-vivo*	[77]
PLA	PLA-PEG (diblock)	thin film	65	*in-vivo*	[49]
	PLA-PEO (star-branch)	solvent evaporation	6-56	*in-vitro*	[48]
	PVA-PEG	solvent evaporation	20-62	*in-vitro*	[50]
	Poly(γ-glutamic acid), galactosamine (targeting)	solvent evaporation	50-54	*in-vivo*	[51]
	PLA-PEG-PLA, PEG-PLA-PEG	solvent evaporation	14-31	*in-vivo*	[52,53]
Chitosan	cholanic acid	dialysis	92	*in-vivo*	[78,194]
	oligomer	dialysis	97	*in-vivo*	[79]
	glyceryl monooleate	emulsion-solvent evaporation	98-100	*in-vitro*	[80]
	mPEG, cholesterol	dialysis	70	*in-vivo*	[81]
	N-acetyl histidine	—	—	*in-vitro*	[82]
	stearic acid, glutaraldehyde	ultrasonication	94-99	*in-vitro*	[83]
Gelatin	—	desolvation	> 80	*in-vivo*	[109-112]
HA	—	desolvation	90	*in-vivo*	[95]
	oligomer	dialysis	—	*in-vitro*	[96]
PBCA	pluronic F127	miniemulsion	80	*in-vitro*	[98]
	chitosan	dialysis	90	*in-vivo*	[99]
	surfactants (dextran 70, cholesterol, PVA, and lecithin)	polymerization	60-80	*in-vitro*	[100]
Albumin	—	high-pressure homogenization	—	*approved*	[88]
	CREKA and LyP-1, peptides (targeting)	—	—	*in-vivo*	[91]
	folic acid (targeting)	desolvation	95	*in-vitro*	[92]
	octaldehyde	dialysis	90	*in-vitro*	[93]
HPG	PEG, PEI	solvent evaporation	—	*in-vivo*	[102]
PEG-PE	EPC, solid triglycerides, cationic Lipofectin lipids	solvent evaporation	~100	*in-vivo*	[103-106]

Table 1: Summary of PX-loaded Polymeric NPs. (*EE=Entrapment Efficiency).

PLGA NPs achieved 10-fold greater bioavailability than Taxol after oral administration [27]. Phospholipids were also used as natural emulsifiers in PLGA NPs, such as 1,2-dilauroylphosphatidylcholine (DLPC) [28] and dipalmitoyl-phosphatidylcholine (DPPC) [29]. Both of the emulsifiers demonstrated greater benefits compared to PVA. Montmorillonite (MMT) was also incorporated into PX-loaded PLGA NPs as both of matrix component and co-emulsifier. The addition of MMT did not change particle size, drug entrapment efficiency, or the *in vitro* drug release from PLGA NPs. Importantly, the PX-loaded PLGA/

MMT NPs enhanced drug cellular uptake over that of pure PLGA NPs by 57-177% and 11-55% in Caco-2 and HT-29 cells, respectively [30]. The PX-loaded PLGA/MMT NPs were further decorated with human epidermal growth factor receptor 2 (HER2) antibodies for targeting purpose, and these targeted NPs exhibited a 12.7-fold enhanced cytotoxicity compared to non-targeted NPs in SK-BR-3 cells [31]. Other targeting ligands, such as RGD [32] and transferrin [33-35], have also been conjugated to PX-encapsulated PLGA NPs for better antitumor efficacy. For example, PX-incorporated PLGA NPs with

transferrin ligand showed 5-fold enhanced cytotoxicity over that of non-targeted NPs or Taxol. The mice treated with targeted NPs demonstrated complete tumor inhibition and significantly prolonged survival compared to all controls after intratumoral injection in a PC3 prostate cancer mouse model [34].

Poly(lactide) (PLA) Nanoparticles

PLA is another widely used matrix material for polymeric NP preparation because of its biodegradable and safe properties. Methoxy poly(ethylene glycol)-poly(lactide) co-polymer (mPEG-PLA) was synthesized and incorporated into the NPs to provide long circulating properties. The *in vitro* cytotoxicity of these NPs increased by 33.3-fold over that of Taxol after 24 h in MCF-7 cells. *In vivo* pharmacokinetic studies demonstrated the AUC and half-life of PX mPEG-PLA NPs in rat plasma were 3.1- and 2.8-fold greater than that of Taxol, respectively [36,37]. PX-loaded NPs with PLA and mPEG-PLA at various ratios of 100/0, 75/25, 50/50, 25/75, and 0/100 were evaluated. It was found that as the mPEG-PLA component in the blend increased, the particle size of NPs and the glass transition temperature of PLA decreased, while the zeta potential of NPs and *in vitro* drug release increased [38]. Co-polymers of PLA/Tween 80 were synthesized and PX-loaded PLA/Tween 80 NPs were shown to be about 3-fold more toxic than PX-loaded PLGA NPs in glioma C6 cells [39]. TPGS was also utilized as an emulsifier in PLA NPs. The Feng group [40] synthesized PLA-TPGS co-polymers using a ring-opening polymerization method. Compared to PX-loaded NPs, the PLA/TPGS NPs showed 1.8- and 1.4-fold enhanced cellular uptake of PX in HT-29 and Caco-2 cells, respectively. The IC_{50} value of PLA/TPGS NPs was also found to be 40% lower than that of Taxol in HT-29 cells [41]. *In vivo* this PX-loaded PLA/TPGS NP formulation achieved a 27.4- and 1.6-fold greater half-life and AUC, respectively, in a xenograft tumor model when compared to Taxol [42]. PX-loaded PLA/TPGS NPs with various ratios of PLA and TPGS were evaluated, and the results demonstrated that the PLA/TPGS ratio had little effect on particle size. However, PLA/TPGS NPs with PLA/TPGS ratio of 89/11 were the optimized formulation in terms of drug entrapment efficiency, cellular uptake, and *in vitro* cytotoxicity [43]. Folate-decorated PX-loaded PLA-TPGS NPs were further formulated to achieve even better therapeutic effect [44,45]. Other targeted PX-loaded PLA NPs, such as HER2 [46], biotin and folic acid [47], were also reported to greatly improve efficacy both *in vitro* and *in vivo*.

PLA co-polymer micelles have also been reported for PX delivery [48-53]. For example, PX-loaded PEG-b-PLA micelles were prepared and the mechanism of action was investigated. It was found that the micelles first interacted with cell membranes and then the loaded PX was released. After that, PX was internalized into the cells by lipid/raft/caveolae-mediated endocytosis pathway. In this way, PEG-b-PLA micelles were able to overcome multiple drug resistance (MDR) which was confirmed by the increased cellular uptake of PX in resistant A2780/T cells. The results also suggested PEG-b-PLA micelles could inhibit P-gp efflux [49]. Paxceed® is a polymeric micelle formulation where PX is encapsulated in PLA-b-mPEG diblock co-polymers. The micellar formulation was found to be more efficacious than Taxol at the maximum tolerated dose (MTD) upon intraperitoneal injection in an MV-522 lung tumor bearing mouse model [54]. Currently, Paxceed® is in phase II clinical trials [55]. Genexol-PM remains the most successful PX micellar formulation to date, which is composed of PLA-b-PEG diblock co-polymers [56]. A preclinical *in vivo* study with Genexol-PM was found to have 3-fold increased MTD and 2-3-fold higher drug concentration in various tissues and more importantly in tumors, compared to Taxol in nude mice. The *in vivo* antitumor

efficacy of Genexol-PM was also significantly improved [57]. In phase I clinical studies, the MTD dose was determined to be 180 mg/m². The plasma AUC and C_{max} increased by 3- and 4-fold, respectively, when the dose increased from 80 to 200 mg/m² [58], which suggested the pharmacokinetics of Genexol-PM were dose-proportional. In phase II clinical studies, Genexol-PM was found to be safe and effective in patients with metastatic breast or advanced pancreatic cancer [59,60]. Phase III clinical studies are currently in process.

Triblock co-polymers of PLA-PEG-PLA and PEG-PLA-PEG were synthesized as carriers for PX. The results demonstrated that the drug release from PEG-PLA-PEG micelles was slower than from PLA-PEG-PLA micelles, and PEG contents in micelles influenced the stealth properties of the micelles. Both of micelles showed 4-fold decreased monocyte cell uptake compared to PLA micelles [52,53]. In another study, a four-armed (star-branched) co-polymer of PLA and PEO was synthesized. Compared to di- and tri-block co-polymers, the star-branched micelles exhibited better controlled and more complete release manner over 2 weeks. Furthermore, the star-shaped micelles had smaller particle size which had the potential to take more advantages of the EPR effect in cancer therapy [48].

In addition to PEG-modified PLGA micelles, PX-incorporated PVP-b-PLA micelles were prepared by Gaucher et al. [50] by an o/w emulsion solvent evaporation method. The cryoprotectant property of PVP allowed the same particle size upon reconstitution after lyophilization, while PEG-modified PEG-b-PLA micelles did not. For targeting purpose, a galactosamine targeted PX-loaded micelle formulation composed of poly(γ-glutamic acid) and PLA was developed. The targeted NP formulation showed the most significant antitumor efficacy compared to other controls and importantly more drug accumulation in tumors was observed in hepatoma tumor-bearing nude mice [51].

Poly(ε-caprolactone) (PCL) Nanoparticles

Deshpande et al. [61] developed poly(ethylene oxide)-modified poly(ε-caprolactone) (PEO-PCL) NPs for co-delivery of PX and C_6-ceramide (an apoptotic signaling molecule) to overcome MDR. The prepared PEO-PCL NPs had high drug entrapment efficiency of >95% with PX and C_6-ceramide drug loading of 10% (w/w). The particle size of the NPs was ~270 nm in diameter. In resistant human ovarian cancer SKOV3$_{TR}$ cells, PX and C_6-ceramide loaded PEO-PCL NPs showed 100-fold enhanced cytotoxicity compared to free PX [62]. *In vivo* PEO-PCL NPs demonstrated remarkable tumor growth inhibition in both wild-type SKOV3 and resistant SKOV3$_{TR}$ xenograft mouse models compared to all the controls. The results indicated the combination of PX and C_6-ceramide incorporated into PEO-PCL NPs overcame MDR in ovarian cancer [63]. The combination of PX and tamoxifen loaded PEO-PCL NPs was also evaluated both *in vitro* and *in vivo*. *In vitro* this formulation lowered the IC_{50} by 10- and 3-fold in SKOV3 and SKOV3$_{TR}$ cells, respectively, when compared to free PX. The *in vivo* PEO-PCL NPs significantly enhanced antitumor efficacy and no acute toxicity was observed [64]. Later, polymeric NP systems for the co-delivery of both PX and P-gp silencing siRNA were developed. In order to do that, poly(ethylene oxide)-modified poly(β-amino ester) (PEO-PbAE) and PEO-PCL NPs were formulated to encapsulate P-gp silencing siRNA and PX, respectively. The co-administration of P-gp silencing siRNA-loaded PEO-PbAE NPs and PX-loaded PEO-PCL NPs completely reversed the MDR based on the fact that the similar cytotoxic activity of NPs in both sensitive SKOV3 and resistant SKOV3$_{TR}$ cells [65].

Similarly, PEG-PCL polymeric micelles were prepared by a co-

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