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EXPERT REVIEW

Polymeric Micelles: Recent Advancements in the Delivery of Anticancer Drugs

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ABSTRACT Nanotechnology, in health and medicine, extensively improves the safety and efficacy of different therapeutic agents, particularly the aspects related to drug delivery and targeting. Among various nano-carriers, polymer based macromolecular approaches have resulted in improved drug delivery for the diseases like cancers, diabetes, autoimmune disorders and many more. Polymeric micelles consisting of hydrophilic exterior and hydrophobic core have established a record of anticancer drug delivery from the laboratory to commercial reality. The nanometric size, tailor made functionality, multiple choices of polymeric micelle synthesis and stability are the unique properties, which have attracted scientists and researchers around the world to work upon in this opportunistic drug carrier. The capability of polymeric micelles as nano-carriers are nowhere less significant than nanoparticles, liposomes and other nanocarriers, as per as the commercial feasibility and presence is concerned. In fact polymeric micelles are among the most extensively studied delivery platforms for the effective treatment of different cancers as well as non-cancerous disorders. The present review highlights the sequential and recent developments in the design, synthesis, characterization and evaluation of polymeric micelles to achieve the effective anticancer drug delivery. The future possibilities and clinical outcome have also been discussed, briefly.

KEY WORDS cancer · hydrophilic corona · hydrophobic core · nanotechnology · polymeric micelles

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ABBREVIATIONS

AA	Acrylic acid
ATRA	All Trans Retinoic Acid
AUC	Area under the curve
CMC	Critical Micellar Concentration
DEHP	Di-(2-ethylhexyl) phthalate
DSPE	Distearoylphosphatidylethanolamine
EPR	Enhanced Permeation and Retention
HA	Hyaluronic acid
IP	Intraperitoneal
IV	Intravenous
LHR	Low molecular weight heparin-all-trans-retinoid acid
LLC	N-lauryl-carboxymethyl-chitosan
mPEG/MPEG	Methoxy PEG
MTD	Maximum Tolerated Dose
MW	Molecular Weight
NQO1	NADP(H): quinone oxidoreductase 1
OSC	N-octyl-O-sulfate-chitosan
p-(CLco-TMC)	Poly (e-caprolactone-co-trimethylenecarbonate)
PBLA	Poly (β-benzyl-L-aspartate)
PCEC	Poly (ε-caprolactone) -polyethylene (glycol) - poly (ε-caprolactone)
PCL	Poly (ε-caprolactone)
PDENA	Poly (2-(4-vinylbenzyloxy) -N,N-diethylnicotinamide)
PDLLA	Poly (D-L Lactide)
PEG	Polyethylene Glycol
PEO	Polyethylene Oxide
PH	Poly (L-histidine)
PLGA	Poly (DL-lactic-co-glycolic acid)
PLLA	Poly (L-lactic acid)
PM	Polymeric micelle
PMMD	Poly (3 (S)-methyl morpholine-2,5-dione)
PTX	Paclitaxel

PVC	Poly vinyl chloride
SGF	Simulated gastric fluid
SIF	Simulated intestinal fluid
tBA	(t- butyl acrylate)
TPGS	d-α-tocopheryl polyethylene glycol
VBODENA	4- (2-vinylbenzyloxy)-N,N-(diethylnicotinamide)
WHO	World Health Organization

INTRODUCTION

According to the recent facts update from WHO, cancers are among the leading cause of death worldwide, accounting approximately 8.2 million deaths in 2012 (1). Among different cancers, which differs in men and women; lung, liver, stomach, colorectal and breast cancers are responsible for the majority of cancer related deaths each year. Breast cancer is by far the most commonly diagnosed cancer among women and is the principle cause of death from cancers among women, globally. Whereas in case of men, prostate cancer is most commonly diagnosed, however majority of cancer related deaths among men is due to lung cancer. The most alarming data is that about half of the diagnosed cases worldwide are from economically developing countries. Chemotherapy, radiation, surgery and hormonal therapies remain the major treatment modules for cancers today, depending on the stage and severity of a particular type of cancer (2). Chemotherapy, which is mostly preferred subsequently to surgery, in most instances suffers from poor pharmacokinetics and inappropriate bio-distribution resulting into unwanted and toxic side effects. In addition to this hydrophobic nature and low molecular weight of anticancer drugs poses problems such as large volume of distribution, short circulation time and less specificity to target site as well as higher exposure and localization at the healthy tissues (leading to side effects such as increased toxicity and adverse effects). Poor solubility and hydrophobicity contributes to a major hurdle in this regard. Therapeutic drugs are transported through the blood; therefore, solubility directly affects absorption and distribution patterns *in vivo*. Copolymers that self-assemble into micelles are being investigated for the last three decades, as vehicles to increase the solubility and decrease the toxicity and unwanted exposure of hydrophobic drugs, especially anticancer drugs. Several novel polymeric approaches have been reported to solubilize and deliver hydrophobes e.g. polymeric micelles, nanoparticles, dendrimers, complexation etc. Much attention, however, has been given to the delivery of anticancer drugs due to the complexity and severity of cancers. In this aspect, polymeric micelles have been reported equally among the other novel nano-carriers.

Polymeric micelles belong to the class of amphiphilic copolymers which aggregates to form nano-scale (1–200 nm) assemblies. These co-polymeric assemblies are composed of two individual functional sections i.e. "inner core or core" and "outer shell or corona" (Fig. 1). In most instances, the outer shell is mainly composed of hydrophilic block such as polyethylene glycol (PEG) whereas the core is usually composed of hydrophobic block which varies enormously. The outer shell controls the *in-vivo* pharmacokinetic properties, while the inner core is responsible for drug entrapment, stability and drug release characteristics. Uniqueness of the polymeric micelles exist in that; these co-polymeric structures can encapsulate hydrophobic drugs in the core while the surface can be modified/engineered or tailored accordingly to achieve the desired properties. Therefore the role of polymeric micelles in drug delivery and targeting becomes promising and opportunistic. Table I summarizes various drugs delivered and reported using different copolymer based polymeric micelles. The present review is aimed to concisely review the studies related to applications of polymeric micelles in anticancer drug delivery and targeting; more systematically and in a step by step manner, compared to existing reviews (31–33). Though several reviews on polymeric micelles has been reported in the past (31–33) however, our efforts were to quote the year wise development of reports for various anti-cancer drugs using different polymeric micelles.

POLYMERIC MICELLES AND EPR EFFECT

Passive targeting approach to solid tumors through size based enhanced permeation retention (EPR) effect plays a pivotal

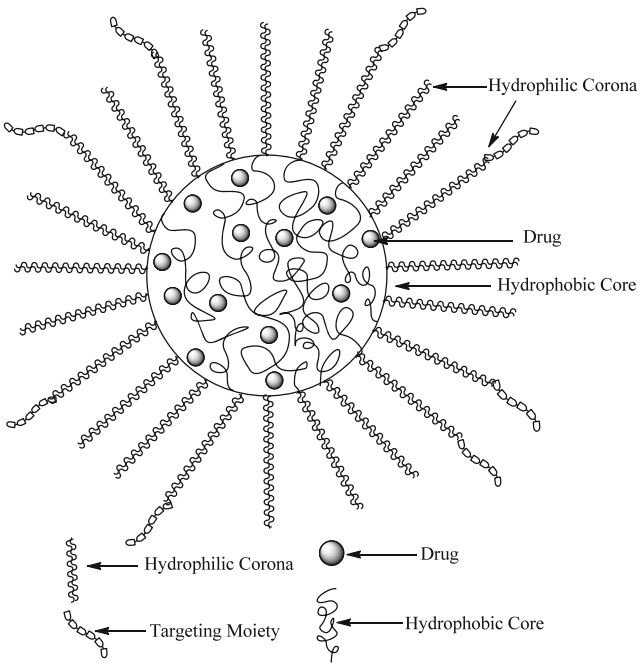

Fig. 1 A typical structure of polymeric micelle representing the drug encapsulated and targeting moiety attached.

Table I Different Anticancer Drugs Delivered Through Polymeric Micelles

Drug	Polymeric micelle used	Average size (nm)	CMC	Loading efficiency %	Cancer cell line	IC_{50} values (approx.)	References
DOX	Gal galactosylated –CHGC cholesterol modified-glycol chitosan	387 ± 29.1	0.586 lg/ml,	71.6	HepG2	407 μg/ml	(3)
	PLGA- PEG	61.48 ± 7.17	0.1 mg/ml,	99.09	HepG2		(4)
	mPEG - PLLA poly (L-lactide)	89.1 ± 11.6	1.3 μg/ml		HSB-2	10–50 μM	(5)
	PEG- PLA	188.43 ± 8.96	9.55 μg/ml.		SKOV-3	4.65 μg/ml	(6)
	PEG- - PBLA	50–70		50–60	C26		(7)
	PEG–polycaprolactone	20	0.98 μg/mL	56.7	K562/ADR		(8)
	PEO- poly (ethylene oxide)- PBLA	20–30		65			(9)
	polyHis/PEG-folate and PLLA/PEG-folate	55–70		75–85	MCF-7		(10)
	Poly (L-histidine)-b-PEG-PLLA- poly (L-lactide)	153 ± 15		90 ± 5			(11)
	PEG- pAsp- poly aspartate			65			(12)
		40			P388 C26, M5076, MX1		(13)
PTX	PEG- poly (ethyleneglycol)750- p-(CLco-TMC)- block-poly (e-caprolactone-co- trimethylenecarbonate)	23.9 ± 0.6	0.002 mg/ml		HeLa	10.6–17.6 μg/ml	(14)
	mPEG-PLLA- poly (L-lactide) PMMD- poly (3 (S)-methyl-morpholine-2,5-dion	70–90	0.0025 g/L	14.3	A-549 and HCT-116		(15)
	P188/LHR and F127/LHR	140			MCF-7	0.281 & 0.273 μg/ml	(16)
	P123/f127	25		90	A549 and SPC-A1	0.1 μg/ml	(17)
	PEG- PDLLA- poly(D, L-lactide)				MCF 7 OVCAR 3	0. 002(IC70) μg/ml	(18)
	PEC-poly (ε- caprolactone)– PEG	20.5		4.8	HEK293 and G6		(19)
	pHPMAmDL -poly(N-(2-hydroxypropyl) methacrylamide lactate)-b- PEG	64	0.0059%		B16F10		(20)
	PDENA – poly (2- (4-vinylbenzyloxy)-N,N-diethylnicotinamide)-PEG-Poly ethylene glycol	100–120	0.036 mg/ml				(21)
	PEG2000–phosphatidyl ethanolamine TPGS- d-α-tocopheryl PEG1000 succinate	15.3			Caco-2		(22)
	FA folic acid-HA-C_{18} hyaluronic acid	184.8–379.7	10 μg/ml	97.3	MCF-7 and A549		(23)
	Chitosan-TOS α-Tocopherol succinate	78	0.0058 mg/ml		MCF-7		(24)
	mPEG-PCL poly (q-caprolactone)	<150		39.58	MCF-7 and HeLa 229		(25)
	VBODENA- 4- (2-vinylbenzyloxy)-N,N-(diethylnicotinamide)-AA- Acrylic acid		31–86 μg/ml				(26)
	mPEG- PDLLA- poly (D, L-lactide)		0.007 mg/ml				(27)
CPT	PEG-DPSE distearoyalphasphatidylethanolamine	230	12.1 μM		KB		(28)
	PEG–PBLA	60–110					(29)
LAP	PEG –PLA	29.6		41.9	DU-149 MDA-MB-231H596		(30)

CMC Critical Micellar Concentration, *DOX* Doxorubicin, *CPT* Camptothecin, *PTX* Paclitaxel, *LAP* β-Lapachone, *PEG* Polyethylene glycol, *mPEG* Methoxy PEG, *PLGA* Poly (D L-lactic-co- glycolic acid), *PBLA* Poly (β benzyl-L-Aspartate), *PLA* Poly (D, L-Lactide)

role to deliver drugs directly to the tumor site. At first, it seemed as receptor-mediated targeting was the only workable way to improve tumor selectivity, and thus many researchers sought to develop conjugates bearing tumor-specific ligands, antibodies or peptides (34, 35). However, more recent studies have shown that polymer-conjugated drugs, nanoparticles, polymeric micelles shows prolonged circulation of the blood and accumulate passively in tumors, even in the absence of targeting ligands (34), suggesting the existence of a passive retention and targeting mechanism. Tumor vasculature usually has a high proportion of proliferating endothelial cells, increased tortuosity, pericyte deficiency and aberrant basement membrane formation. This defective vascular structure, which is likely the result of rapid vascularization necessary to provide oxygen and nutrients for fast-growing cancers, decreases lymphatic drainage and renders the vessels permeable to macromolecules. Because of the decreased lymphatic drainage, the permeant macromolecules are not removed efficiently, and are thus retained in the tumor. This passive targeting phenomenon, first identified by Maeda *et al.* (36, 37) is called as EPR effect (Fig. 2). Since this first identification, numerous studies have shown that the EPR effect results in passive accumulation of macromolecules and nano-sized particulates (e.g. polymer conjugates, polymeric micelles, dendrimers, and liposomes) in solid tumors, increasing the therapeutic index while decreasing the undesirable side effects. The optimum size of nano-carriers that can be accumulated in a tumor by the EPR effect is not yet precisely known, however, studies using liposomes and nanoparticles have indicated that the cut off size of the pores in tumor vessels is as large as 200 nm to 1.2 mm (38, 39), and direct observation of tumor vasculature has demonstrated a tumor dependent pore cut off size ranging from 200 nm to 2 mm (40, 41). The suitable polymeric micelles size, too large for extravasation from normal vessel walls and renal excretion, and too small for extravasation from tumor blood vessels, combined with the pathophysiological characteristics of solid tumor tissues, hyper-vascularity, incomplete vascular architecture, secretion of vascular permeability factors and the absence of effective lymphatic drainage leads to the EPR effect of polymeric micelles in solid tumors (12, 42). This warrants the passive targeting of polymeric micelles, which is the basis of active targeting.

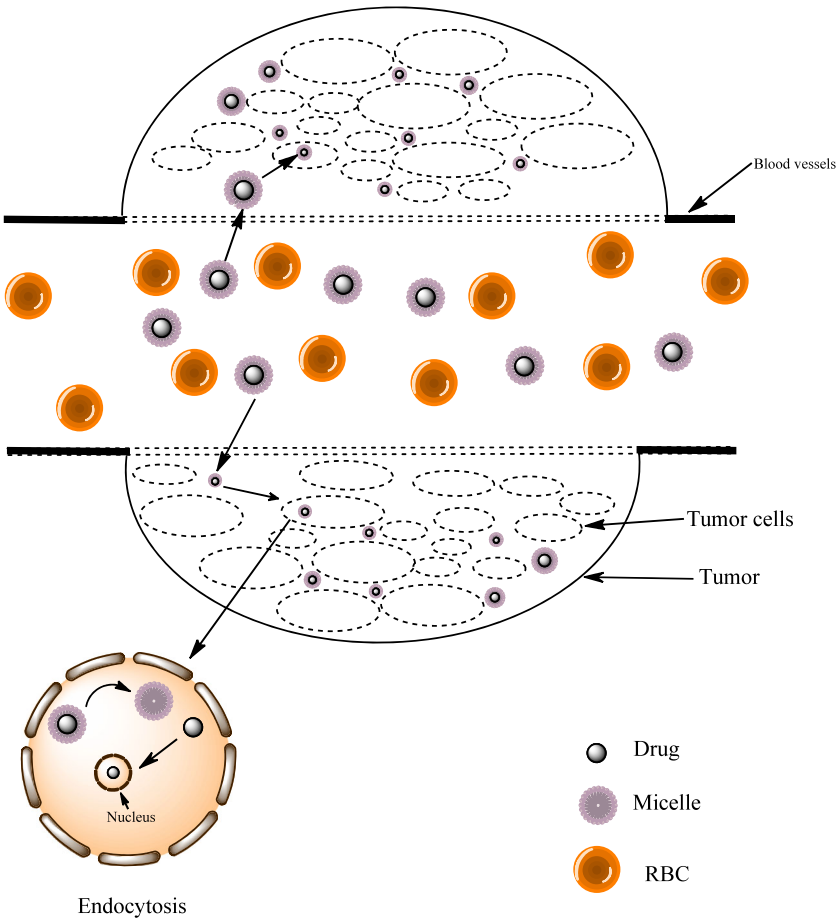

Fig. 2 Enhanced permeation and retention effect in relation to polymeric micelle delivery in cancerous cell.

STRUCTURE: THE "CORE" AND "CORONA"

The structure of polymeric micelles follows and exemplifies the similar structure of micelles proposed as per different miceller theories. It is comprised of a "core", which is usually a hydrophobic section while the exterior, which is also known as "corona", represents a hydrophilic block of the copolymer structure (Fig. 3). In the past two decades, several different polymers have been reported to play the role as a "core" or "corona" with their own added merits which has been utilized extensively in drug delivery and targeting. The following paragraphs would comment on the different types of polymers used for hydrophilic and the hydrophobic block of a polymeric micelle.

Corona: The Hydrophilic Block

Hydrophilic block represents the surface or surface functionality of the polymeric micelles. In most instances it has been observed that hydrophilic block in polymeric micelles is almost exclusively PEG. PEG being FDA approved and with meritorious properties are one of the most extensively used hydrophilic coronas in polymeric miceller delivery (Fig. 3). The commendable properties of PEG such as excellent biocompatibility, stealth nature in blood circulation, low molecular weight and availability of a large number of hydroxyl groups, non-toxic nature makes PEG an excellent corona for encapsulation and solubilization of hydrophobic drugs and other bioactives. The hydrophilic shell of PEG, surrounding the micellar core can protect undesirable phenomena, such as inter-micellar aggregation or precipitation, protein adsorption, and cell adhesion (43, 44). In context to anticancer drug delivery using polymeric micelles, PEG has been used as a hydrophilic block for the delivery of drugs such as doxorubicin (DOX), paclitaxel (PTX) as well as camptothecin (CPT) and β lapachone etc. (5, 8, 13, 18, 20, 25, 29, 30, 45). In the majority of reports, researchers have largely used low molecular weight (~2000–5000 Da) PEG for the preparation of amphiphilic block copolymeric self-assemblies (25, 29, 46). However, in some of the report's researchers have also used PEG of 12, 000 Da as molecular weight (25, 29, 46). The use of different molecular weights PEG may have the impact of critical micellar concentration (CMC) and drug loading efficiency of the developed polymeric micelles. In an excellent study reported by the Ping *et al.* in 2009, the authors developed mPEG (methoxy PEG) conjugated N-octyl-O-sulfate chitosan

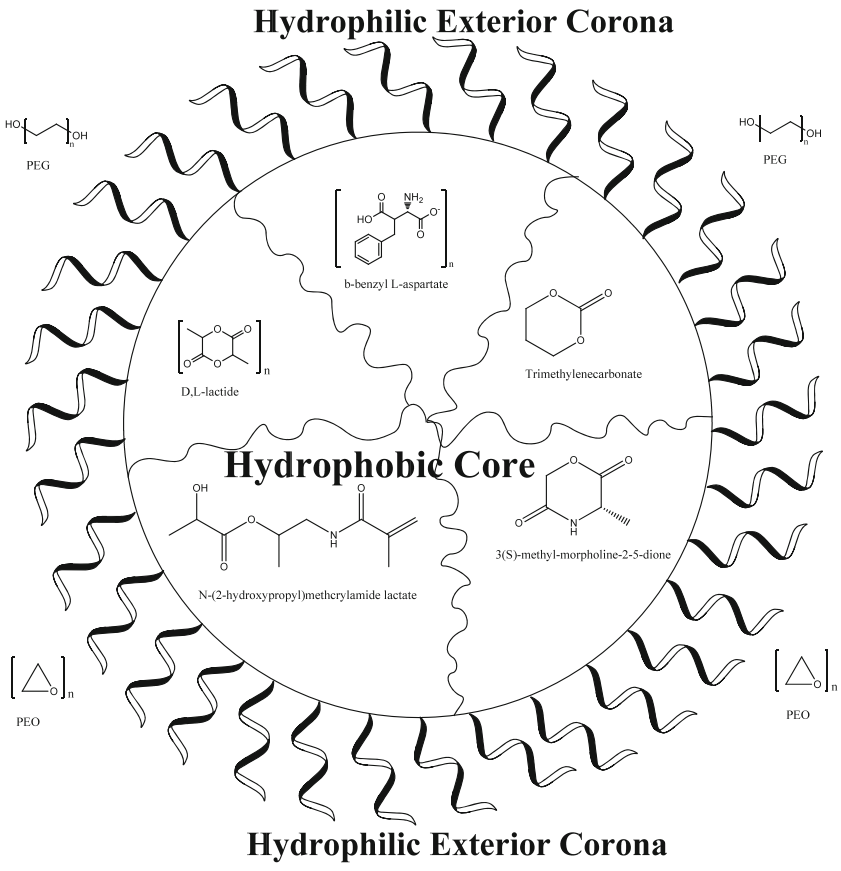

Fig. 3 Examples of polymers used as hydrophilic corona and hydrophobic core of the polymeric micelles.

PEG: Polyethylene Glycol
PEO: Polyethylene Oxide

polymeric micelles for the delivery of PTX using three different molecular weights of mPEG i.e. 1100, 2000 and 5000. It was observed that the introduction of PEG groups decreased the adsorption of plasma protein to micelles (46). The effect of polymeric micelles having a PEG of 2000 and 5000 Da was stronger than the corresponding 1000 Da PEG based micelles. Also the entrapment efficiency of PTX was found to be highest in case of 2000 Da PEG (>82%) which itself explains the importance of PEG as corona. The biocompatible nature, lesser toxicity and multiple hydroxyl groups equally contribute in selecting an appropriate hydrophilic block for the development and characterization of polymeric micelles. PEG fits the best in this class of polymers to be used as corona for the polymeric micelles. In addition to PEG, however, researchers have also used poly (ethylene oxide) or PEO as a hydrophilic block in some reports (9).

Core: The Hydrophobic Block

Unlike hydrophilic block, the use of hydrophobic block (Fig. 3) varies enormously as evidenced by the past literature (4, 5, 7–10). The design and choice of the hydrophobic block can be tailored accordingly with a wide variety of lipophilicity and structures based on the desired properties of polymeric micelles. However, for the anti-cancer drug delivery using polymeric micelles, the majority of researchers has used poly (D,L-lactide), poly (β-benzyl-L-aspartate; PBLA), polycaprolactone (PCL) and poly (DL-lactic-co-glycolic acid; PLGA) etc. as core (4, 5, 7–10). Chemically conjugated DOX with biodegradable di-block copolymer composed of poly (L-lactic acid) (PLLA) and methoxy-poly (ethylene glycol) (mPEG) was reported in the year 2002. PLLA was used as hydrophobic block or core of the developed polymeric micelles (5). Yin and Bae (2009) reported physicochemical aspects of DOX loaded pH sensitive polymeric micelles. The researchers used a mixture of poly (L-histidine) -b-PEG/poly (L-lactide) -b-PEG (PH-PEG and PLLA-PEG) (75/25, *w*/*w*), PLLA acted as the inner hydrophobic core for micelles (11). Li *et al.* (2012) prepared a polydepsipeptide containing triblock copolymer as self- assembling polymeric micelle for PTX delivery. The author synthesized biodegradable polydepsipeptides based novel triblock copolymers, poly (ethylene glycol) -poly(l-lactide) –poly(3(S)-methyl-morpholine-2,5-dione) (mPEG–PLLA–PMMD) for PTX delivery through polymeric micelles (15). The above examples of hydrophobic polymers clarifies that the choice and selection of core have represented and displayed more room in terms of core options, for the synthesis of copolymers to achieve effective encapsulation and desired delivery of anticancer drugs through polymeric micelles compared to hydrophilic block.

ANTICANCER DRUGS DELIVERED THROUGH POLYMERIC MICELLES

Paclitaxel (PTX)

Taxol, which is popularly known as paclitaxel (PTX), has been successfully used in the past, for several years, in the treatment of many cancers such as lung, ovarian, breast, head and neck cancer as well as advanced forms of Kaposi's sarcoma (47, 48). PTX is a natural diterpenoid, which is isolated from *Taxusbrevifolia* bark. PTX exerts its effect by inducing apoptosis through binding to microtubules within a dividing cell during mitosis, leading to kinetic stabilization and thereby preventing cell division through mitotic arrest (49). Poor aqueous solubility of PTX (0.6 μg/ml) has been one of the major hurdle in the development of a successful formulation to achieve safe and effective formulation of PTX (50). Commercially, PTX is usually delivered in a mixture of Cremophor® EL (polyethoxylated castor oil) /absolute ethanol (50:50 *v*/*v*). However, it has been reported that Cremophor EL causes many side effects such as hypersensitivity reactions, myelosuppression and neurotoxicity (51, 52). Therefore the majority of researchers has attempted towards developing aqueous based formulations for Taxol. In the recent past, Taxol has been delivered using liposomes, nanoparticles, dendrimers and other polymeric carriers such as niosomes, nanoemulsions (53–59). Polymeric micelles, with inbuilt unique features to solubilize insoluble drugs, has been one of the preferred choices for the researchers (Fig. 5). Currently, an mPEG–PLA based biodegradable co-polymeric micelle encapsulated PTX system (Genexol®-PM) is under phase II clinical trial in Korea and US. Additionally, NK105 reported by Matsumara *et al.* is also under clinical trial phase II (60) which shows the commercial potential and opportunities with this carrier in drug delivery and targeting. The clinical outcome of the polymeric micelles of other drugs has been further discussed in the further section of the article in more detail.

PTX Solubilization and In Vivo Assessment

In one of the earliest attempts for PTX delivery using polymeric micelles, Zhang *et al.* (1996) developed amphiphilic diblock co-polymeric micelles composed of poly (DL-lactide) -methoxy poly (ethylene glycol) for PTX (61). The CMC of the developed polymeric micelles was measured by using fluorescence techniques. Enhanced solubilization of PTX was reported in the study using the above mentioned polymeric micelles. In a further separate study the anti-tumor activity and bio-distribution of these polymeric micelles was investigated *in vitro* and *in vivo* (62). The authors reported the *in vitro* cytotoxicity studies of both polymeric micellar PTX (PDLLA-MePEG 2000-40/60 or 2000-50/50) and compared to marketed formulation Cremophor, which showed the same

efficacy in inhibiting the growth of Hs578T breast tumor cells, SKMES non-small-cell lung tumor cells, or HT-29 colon tumor cells. *In vivo* studies were performed on B6D2F1 mouse model, bearing P388 leukemic tumor treated intra peritoneally (IP) with developed polymeric micelles and Cremophor, separately. The PTX micellar formulation was found to be five fold more tolerated as compared to Cremophor PTX. In addition, micellar PTX displayed improved and more efficacious *in vivo* anti-cancer activity in murine P388 leukemia model of malignancy in comparison to Cremophor PTX at their maximum tolerated doses (MTD) (62). In yet another *in vivo* study of MePEG-PDLLA micelles of PTX, Zhang *et al.* (1997) administered Cremophor EL and polymeric micelles based formulations (IV and IP) to nude mice with MV-522 lung carcinoma. MePEG-PDLLA based PTX was observed to be more efficacious IP (98.7% tumor growth inhibition) than Cremophor PTX IP (83.0% tumor growth inhibition) at their MTDs. The study also reported the biodistribution behavior of micelles (63). In another study related to MePEG-PDLLA, Burt *et al.* (1999) further synthesized a range of diblock copolymers containing one block of MePEG and one block of either PDLLA or copolymers of poly (D, L-lactide-co-caprolactone) (PDLLACL) or poly (glycolide-co-caprolactone) (PGACL) to study the stability and other properties of PTX loading. The outcome of the study proved that among all the considered micelles, MePEG-PDLLA co-polymeric formulation was most stable physically and displayed the highest PTX solubilization properties. The study also reported the *in vitro* and *in vivo* biocompatibility/toxicity studies in animal models and reported that the MePEG-PDLLA micelles were biocompatible and non-toxic (64). Stability studies of PTX loaded MePEG-PDLLA micelles were further studied by Kim *et al.* In this study Kim *et al.* investigated leaching properties of MePEG-PDLLA micelles as a property for stability assessment in comparison to clinical PTX formulation i.e. Taxol (27). Taxol, which is 1:1 ratio of Cremophor EL and dehydrated ethanol, is known to leach di-(2-ethylhexyl) phthalate (DEHP) from polyvinyl chloride (PVC) infusion bags and PVC administration sets (65). DEHP is suspected to be a hepatotoxin, carcinogen, teratogen and mutagen (66). Results confirmed that Taxol-vehicle contributed the majority of the DEHP extracted from PVC infusion bags and PVC administration sets, while the absence of Cremophor® EL and ethanol in the polymeric formulation significantly reduced the amount of DEHP extracted from PVC infusion bags and administration sets which was the case with the MePEG-PDLLA micelles (27).

Kim *et al.* (2001) designed Genexol-PM polymeric micelle to evaluate pharmacokinetics, tissue distribution, toxicity and efficacy of PTX. The authors developed polymeric micelle using low molecular weight, non-toxic and biodegradable amphiphilic diblock copolymer monomethoxy poly (ethylene glycol) -block-poly (D, L-Lactide) (mPEG-PDLLA). Pharmacokinetic study was reported by two compartment open model, C_{max} was reported for Genexol-PM and PTX 82.83 mg/ml (dose 50 mg/kg) and 94.08 mg/ml (dose 20 mg/kg); respectively. The authors reported higher maximum tolerable dose of Genexol-PM than Taxol in nude mice. *In vitro* cytotoxicity of Genexol-PM and PTX was similar at the same concentration against MCF-7 and OVCAR3 cell lines. Bio-distribution of PTX after administration of Genexol-PM was 2–3 folds higher in spleen, liver, lungs, kidney, heart and in tumor than of PTX. The LD_{50} of Genexol-PM was also reported higher than that of PTX. *In vivo* study was performed on SKOV-3 human ovarian cancer and MX-1 breast cancer cell bearing nude mice and it was found that the activity of Genexol-PM was 3 folds higher than PTX. Author reported IC_{70} value 0.002 μg/ml for Genexol-PM (18). In the year 2002 Burt and Liggins extensively reviewed the applicability of polymeric micelles especially, MePEG-PDLLA for PTX delivery. The authors focused on the synthetic reaction conditions, which influences the overall polymerization reactions, thermal properties of the polymeric micelles, methods of drug encapsulation in copolymeric matrices and others (67).

In continual exploration of applicability of polymeric micelles for PTX delivery, Hennink *et al.* (2005) developed and characterized PTX loaded thermo-sensitive and biodegradable polymeric micelles consisting of poly (N- (2-hydroxypropyl) methacrylamide lactate) and poly (ethylene glycol) (pHPMAmDL-b-PEG) copolymers. PTX was encapsulated up to 2 mg/ml of polymeric micelles. The developed polymeric micelles were stable for 200 h, at physiological conditions (37°C and 7.4 pH). PTX was 100% solubilized up to 2 mg/ml. The average size of loaded polymeric micelle (64 nm) was similar to empty polymeric micelles (60 nm) with low polydispersity. Internalization of micelle was studied on B16F10 cells and it was found that the micelles were uptaken by cells at 37°C. PTX loaded micelles displayed comparable cytotoxicity and the empty micelles were less toxic than Cremophor EL. Though the study did not report the *in vivo* applications, however the *in vitro* applications were very promising (20).

Preat *et al.* (2009) reported new self-assembling poly (ethylene glycol) 750-block-poly (ε-caprolactone-co-trimethylene carbonate) [PEG-p-(CL-co-TMC)] polymeric micelles for PTX delivery. PEG-p-(CL-co-TMC) forms micelles spontaneously in aqueous medium without the need of dialysis or addition of organic solvent. The copolymer does not require any dialysis and evaporation for loading of the drug and forms micelle only by gentle mixing. Aqueous solubility of PTX was increased from 1 μg/ml to 1.82 mg/ml which was a three order fold increase. They reported slower release of PTX from micelle than Taxol. There was no significant difference in cytotoxicity of micelle loaded PTX and Taxol. *In vitro* cytotoxicity of the PEG-p-(CL-co-TMC) micelles was less compared to Cremophor EL. MTD of PTX-loaded micelles and

Taxol in mice were 80 and 13.5 mg/kg, respectively, after IP administration; and 45 and 13.5 mg/kg, respectively, after IV administration. The cytotoxic activity of PTX loaded micelles was higher due to EPR. *In vivo* antitumor activity against TLT-tumor-bearing mice were found to be comparable than Taxol (14).

Liang *et al.* (2012) reported α-Tocopherol succinate-modified chitosan (CS-TOS) micelles for PTX delivery. The authors incorporated PTX into micelles by probe-type ultrasonic method. The authors investigated *in-vitro* cytotoxicity in MCF-7 cells and observed PTX loaded micelles were equipotent to Cremophor EL preparation. The *in-vivo* cytotoxicity in U14 tumor bearing mice was found to be with better therapeutic effect than Taxol and with low toxic effects (24). Wang *et al.* (2012) prepared polysorbate 80 coated PCEC (poly (ε-caprolactone) –poly (ethylene glycol) -poly (ε-caprolactone)) micelles for PTX delivery. PCEC is a triblock copolymer. They used thin-film hydration for micelle formation. For increasing the loading of the drug in micelle different PTX/PCEC ratios were used. When PTX/PCEC ratio was increased, loading efficiency also increased. At the ratio of 2/98 loading efficiency was decreased due to saturation between PTX and PCL core of the micelle. PTX micelle with PTX/PCEC ratio of 8/92 was used for further studies. Release of the PTX from PTX-PCEC and PTX-PCEC-P80 micelle was in a sustained manner than free PTX. The release pattern was bi-phasic; with initial burst phase for 24 h followed by slow release. Polysorbate 80 coating led to faster release of PTX than uncoated micelles due to solubilization of hydrophobic drugs. The authors reported that the polysorbated coated PCEC micelles were less toxic according to evaluation of HEK293 cells *in vitro*. PTX-PCEC-P80 micelle had a significant efficiency on G6 glioma cells *in vitro*. PTX-PCEC-P80 micelles significantly increased the uptake of PTX in the brain without any execrable accumulation in other organs in comparison with Taxol and the uncoated micelles (68).

Yoncheva *et al.* (2012) reported a pluronic based micelle for PTX delivery. They stabilized the micelle using argon, irradiation, and high pressure before the loading of PTX. After stabilization, the drug loading capacity was increased by 1–1.5 mg. Plasma pharmacokinetic parameters were observed with oral as well as IV administration of PTX-polymeric micelles and compared with Taxol. The AUC for IV administration of PTX-polymeric micelles was 1.2 fold more than commercial Taxol and bio-availability for oral administration was about 0.9 while commercial Taxol did not show availability in plasma (69).

Zhao *et al.* (2012) prepared a polydepsipeptide containing triblock copolymer as self-assembling polymeric micelles for PTX delivery. They synthesized biodegradable polydepsipeptides based new triblock copolymers, poly (ethylene glycol) –poly (l-lactide) –poly (3 (S)-methyl-morpholine-2, 5-dione) (mPEG–PLLA–PMMD) by ring opening mechanism and characterized as self-assembling micellar system for PTX delivery. They prepared three different triblock using same mPEG and PLLA, of same molecular weight 2000 in each triblock and varying molecular weights of PMMD 700, 1400 and 2800 in different triblocks. They used mPEG2000-PLLA2000-PMMD1400 for further studies. The authors reported high solubilization of PTX in mPEG2000-PLLA2000-PMMA1400 due to stabilization of inner PLLA by PMMD and drug loading efficiency was higher than other systems. Two phase and pH sensitive release of PTX from mPEG2000-PLLA2000-PMMD1400 was reported. At acidic pH triblock based polymeric micelles get shrunk and prevent release of drug while at pH 7.4 it leads to fast release. Cytotoxicity was investigated on A-549 and HCT-116 cells and higher activity along with higher retention was observed (70). In the most recent study, Šmejkalová *et al.* (2014) studied the chemical and physical structure of PTX. They prepared micelle by using hyaluronic acid (HA). They grafted HA with C6 or C18:1 acyl chain and they got 70% loading efficiency of PTX. The authors reported PTX was changed into an amorphous state from crystalline state and cytotoxicity was higher in this isomeric form. The study reported the importance of different isomers on the cytotoxic behavior of PTX (71).

Chitosan Based Polymeric Micelles for PTX Delivery

Chitosan, a polysaccharide derivative, is a biocompatible, biodegradable and non-toxic polymer which is generally synthesized from N-de-acetylation of chitin (72). Chitosan consists of 2-amino-2-deoxy-(1-4b) -D-glucopyranose residues (D-glucosamine units) and little or no N-acetyl-D-glucosamine units. Chitosan and its derivatives have attracted Researchers more interests in the recent past due to their favorable properties particularly in drug delivery. Chitin-chitosan itself cannot form micelles due to absence of amphiphilicity. However, researchers have attempted in the past to develop chitosan or chitosan derivative based micelles for delivering anti-cancer drugs such as Camptothecin and PTX (73, 74). In the year 1998, Miwa *et al.* developed *N*-lauryl-carboxy methyl-chitosan (LLC) based polymeric micelles for PTX solubilization and to achieve reduced toxicity compared to Taxol. Therefore the lauryl groups were attached to chitosan to impart hydrophobicity, while the hydroxyl groups were attached with carboxymethyl moiety to impart the hydrophilic nature. The authors reported 1000 fold increase in aqueous solubility for PTX using these polymeric micelles. The developed LLC polymeric micelles were safer as evidenced by hemolysis studies and were found to be more effective in cytostatic activity against KB cells. The study was among the first studies utilizing chitosan derivatives for the preparation of polymeric micelles (75). In the later years, Zhang *et al.* reported various micellar studies which utilized chitosan derivatives by attaching sulfates and

alkyl groups for PTX delivery (73, 76–78). Zhang *et al.* (2003) synthesized a series of *N*-alkyl-*O*-sulfate derivatives of chitosan using different chain lengths of alkyl group ($n=8$, 10, 12). The sulphate group attached to chitosan was responsible for hydrophilicity while the alkyl group was responsible for the hydrophobic nature of the polymeric micelles. Though the size of the developed PTX loaded *N*-alkyl-*O*-sulphate chitosan based micelles was larger, however the developed micelles behaved excellent solubilizers for PTX in water. Especially the *N*-octyl-*O*-sulphate chitosan based micelles (OCS) were able to solubilize PTX up to 2.01 mg/ml in water. OCS 1 was selected for further studies by Zhang *et al.* in yet another study (77). In this study, the authors extensively characterized OCS for the stability, *in vitro* release, CMC and other physical properties. The authors reported 25% *w/w* loading of PTX in the micelles. The *in vitro* release of PTX was found to be slow and sustained and the developed PTX loaded micelles were more stable. Developed PTX-loaded OCS micelles were further evaluated for pharmacokinetics, bio-distribution, efficacy and safety in tumor bearing mice models (73). The authors extensively studied the *in vivo* antitumor effect in Sarcoma180, Ehrlich solid carcinoma, Hepatoma solidity, Lewis lung cancer mice models and compared with the Taxol. It was found that both the formulations had similar antitumor activity at 10 mg/kg dose, but the PTX-loaded OCS micelles were safer than the commercial PTX formulation Taxol. The same group of researchers further reported biocompatibility evaluations, such as acute toxicity, injection irritation; anaphylaxis, hemolysis and cell viability studies of PTX loaded *N*-octyl-*O*-sulphate chitosan micelles in rat model through an IV and IP route. The LD_{50} values of PTX loaded *N*-octyl-*O*-sulphate chitosan micelles administrated by IV and IP were calculated as 102.59 and 130.53 mg/kg, respectively. The study was performed using FITC and tritium labeling methods. Pharmacokinetics studied in much detail revealed that almost 75% of the dose was excreted through urine over a period of 7 days time and was the predominant way of excretion (78).

In a recent study by the same group of researchers, Ping *et al.* (2009) reported a further modification of *N*-octyl-*O*-sulphate chitosan micelles through PEG (mPEGOSC) for PTX delivery. Authors synthesized nine different chitosan derivatives with different molecular weight and degree of substitution (DS low, middle, and high) of PEG and chitosan. They compared pharmacokinetic parameters with PTX loaded micelle based on OSC (PTX-OSC) and found AUC (area under curve) of PTXmPEGOSC2000M was higher than PTX-OSC and lower than Taxol. Tissue distribution of PTXmPEGOSC200M was higher than PTX-OSC. PTX-OSC micelle was highly phagocytized than PTXmPEGOSC2000M by RES system. They reported, micelle based on mPEGOSC200s with high DS (degree of substitution) showed highest loading rate, entrapment efficiency and the smallest particle size. Micelles based on PEGOSC with high DS of chitosan showed slower dissociation when diluted below CMC and showed higher stability in blood. PTX-mPEGOSC2000M had longest mean residence time (MRT) than Taxol in body (46). The above mentioned studies conclusively reported that the PTX loaded *N*-octyl-*O*-sulphate chitosan micelles can be a promising carrier for PTX solubilization and safe for per oral delivery.

Mixed Polymeric Micelles for PTX Delivery

In a different set of studies mixed micelles were reported for the effective solubilization of PTX. Gao *et al.* (2002) reported the solubilization of drugs such as Taxol and tamoxifen using diacyllipid-PEG-phosphatidyl ethanolamine micelles as nanocarriers (79). In their further study in 2003, Gao *et al.* reported mixed miceller delivery of Taxol prepared from PEG-distearylphosphoethanolamine (PEG2000-PE) and egg phosphatidyl choline. The developed mixed micelles were conjugated to the antibody at the micellar surface. It was observed that the Taxol loaded mixed micelles were more cytotoxic when studied against cancer cell line model compared to control (80). Dabholakar *et al.* (2006) prepared mixed micelle consisting of PEG 2000–phosphatidyl ethanolamine conjugate (PEG2000–PE) and D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) in 1:1 molar ratio. The solubility of PTX was increased by the addition of TPGS while solubility was less in PEG2000-PEG micelles. Drug loading was higher in PEG2000-PE/TPGS than PEG2000-PE. They reported slow release of PTX and PTX loaded mixed micelle were stable enough till 3 months at 4°C. The authors found that mixed micelles can bypass P_{gp} efflux investigated in Caco-2 cells. Cell internalization (Fig. 4) was reported through rhodamine labelled micelles for the developed polymeric micelles (Fig. 5) (22).

Pluronics has been reported to reverse the multi-drug resistance of certain drugs such as DOX (81). Pluronics based micelles were prepared by Wang *et al.* (2007) using Pluronic P105 and L101 for the solubilization of PTX. The same micelles were further surface engineered with folate residues for the effective internalization in drug resistant cells (82). Same researchers further explored the bio-distribution and pharmacokinetic parameters of PTX loaded P105/L101 mixed polymeric micelles (83). The AUC of these micelles was 4.9 folds higher than Taxol injection *in vivo*. In a similar attempt, Fang *et al.* (2009) reported pluronic P123/P127 mixed micelles for PTX and characterized them for various physical properties. The developed mixed micelles were stable with high drug loading, and smaller in size. They observed cytotoxicity against A549 and SPC-A1 cells and observed IC_{50} less than Taxol injection and free Taxol (17). Dahmani *et al.* (2012) reported pluronic/LHR based mixed micelles for oral administration of PTX. Mixed polymeric micelles were comprised of the conjugate of pluronic copolymers and low molecular

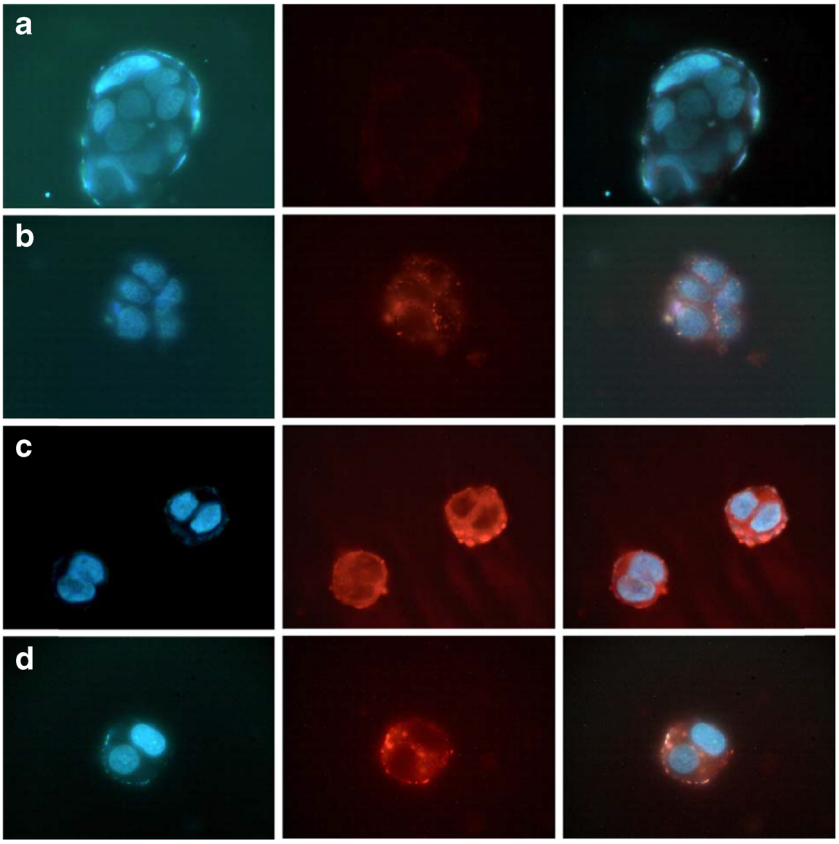

Fig. 4 Internalization of Rh-PE-labeled PTX-loaded PEG2000–PE/TPGS micelles by Caco-2 cells following fluorescence microscopy. *Left panel* (blue fluorescence) represents nuclear staining of Caco-2 cells using Hoechst 33342, middle panel (red fluorescence) represents the fluorescence from Rh-PE-labeled PTX -loaded mixed micelles associated with Caco-2 cells; the *right panel* represents the composite of the two: 1 h incubation (**a**); 3 h incubation (**b**); 6 h incubation (**c**); 12 h incubation (**d**) (reprinted with permission from Elsevier Publishers Ltd from ref. no (22)).

weight heparin-all-trans-retinoid acid (LHR) conjugates. They designed new mixed polymeric micelles (MPMs) which were prepared using LHR conjugates and pluronic triblock copolymers (two most commonly used pluronics F127 and P188 were used for the formulation) in order to enhance PTX oral bioavailability. They prepared MPMs with different weight ratio of pluronic/LHR (F127/LHR and P188/LHR) and as the ratio increased CMC also increased, and MPMs with 1:4 were selected for further studies. MPMs with a ratio of 1:4 of pluronic/LHR have highest loading efficiency and lowest CMC due to high hydrophilic nature of P188 than F127. They reported, MPMs can be stored for 3 months at room temperature. Release of PTX from MPMs was higher in P188/LHR than F127/LHR due to high hydrophilic nature of P188. The cytotoxic activity of PTX was higher in PTX loaded MPMs due to a higher release of PTX and inhibition of P_{gp} efflux. MCF-7 cells were used for cytotoxic evaluations. Higher cytotoxicity and bioavailability was observed for PTX loaded MPMs (84).

Targeted PTX Delivery Using Polymeric Micelles

The majority of the attempts explained earlier by different research groups were towards the solubility and the stability improvement of PTX in its miceller formulations. However, researchers also tried to target PTX using ligand conjugated polymeric micelles. In one of such approach Park *et al.* (2005a) reported a folate-conjugated methoxy poly (ethylene glycol) / poly (ε-caprolactone) (MePEG-PCL) amphiphilic block copolymeric micelles for targeted delivery of PTX. Author synthesized copolymers with and without folate conjugation i.e. MPEG/PCL (PMSEP50) and MPEG/PCL (PFOL50), respectively using different ratios of hydrophobic/hydrophilic counterparts. The general observation was drug loading increased as the hydrophobic chain length is increased. Loading efficiency was higher in PFOL50 than PMSEP50. Release kinetics were same in both the types of micelles. In the first 36 h release was faster and there after sustained release over 7 days, was observed. *In vitro* cytotoxic effect was investigated against MCF-7 and HeLa 229 cells and it was observed that folate conjugated micelles displayed higher cytotoxicity. The authors also reported the receptor mediated higher uptake for the folate conjugated micellar formulations (25). This was not the only attempt for targeting PTX loaded polymeric micelles. In a recent attempt by Liu *et al.* 2011 hyaluronic acid (HA) derivative grafted with octadecyl (hydrophobic) moiety was further conjugated to folic acid (FA-HA-C_{18}). HA residue was designed to act as hydrophilic part and folate as a ligand at the same time octadecyl component as hydrophobic core. The authors prepared different micelles by varying DS (no of octadecyl group per 100 sugar residue of HA) portion through controlling feed ratio of polymer and drug. The authors

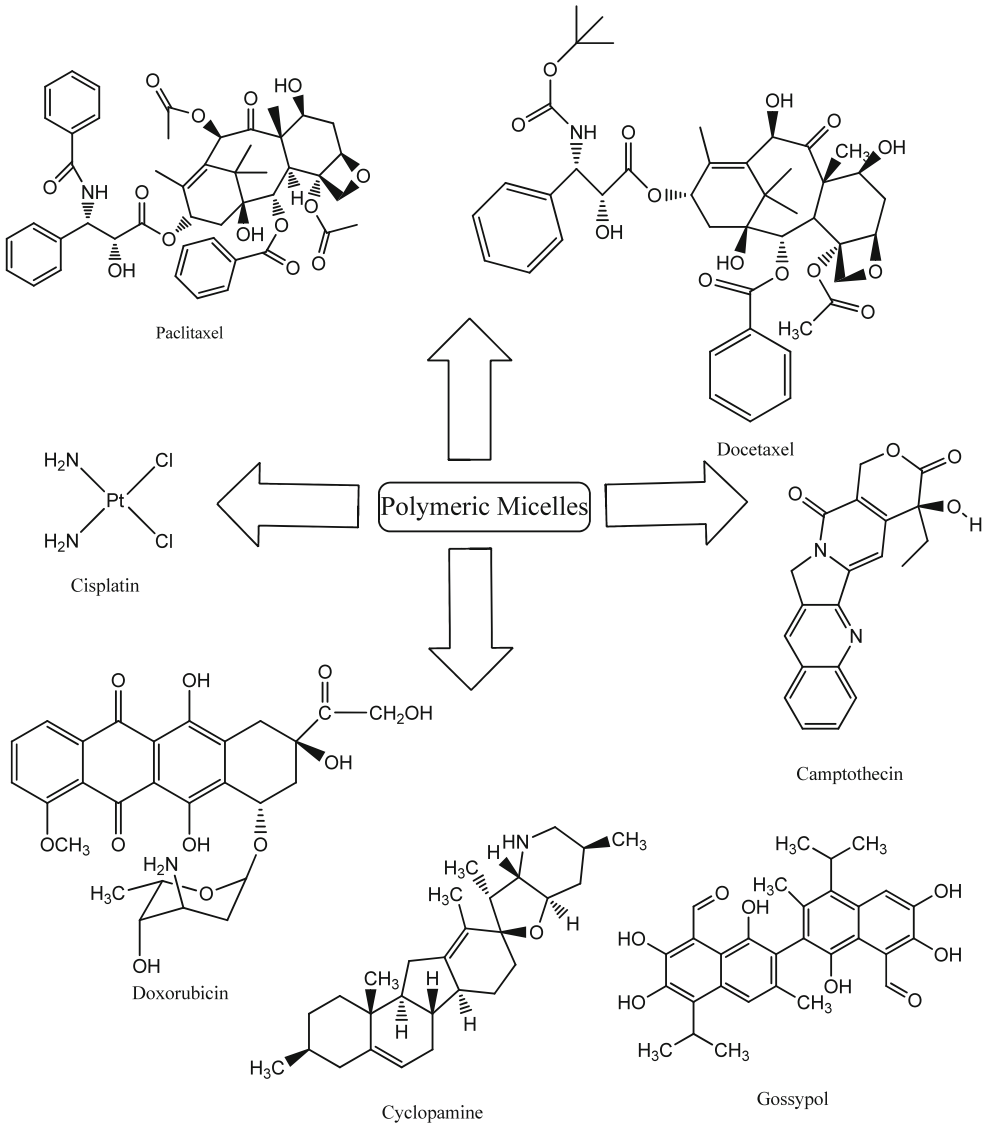

Fig. 5 The chemical structures of the anticancer drugs delivered through the use of different polymeric micelles.

reported that as the DS was increased CMC and particle size were found to be decreased while PTX loading increased. Release of PTX was delayed with high DS. Cytotoxicity was evaluated against MCF-7 and A549 cell lines and it was observed that micelle loaded PTX showed slightly high cytotoxicity due to higher uptake through CD44 receptors (85).

Hydrotropic Polymeric Micelles for PTX

Insolubility, being the major constraint in PTX's formulation development, has been a prime focus by researchers and the majority of attempts made were first to solubilize PTX in water. In one of such attempts, hydrotropic principles were used for PTX solubilization through polymeric micelles. Hydrotropy is a collective molecular phenomenon describing an increase in the aqueous solubility of a poorly soluble compound by the addition of a relatively large amount of another solute (i.e., a hydrotrope). The clear mechanism of hydrotropy has not been reported yet, however the added solutes are believed to be responsible for the solubility enhancement of the hydrophobic drug candidate. In one of such attempts Park *et al.* (2005b) synthesized a hydrotropic polymeric micellar system for PTX delivery using hydrotropic polymer based on N, N-diethylnicotinamide. Authors synthesized three different block co-polymericpolymeric micelles i.e. poly (d, l-lactide) -PEG (PLA-PEG), and poly (phenylalanine) -PEG (PPA-PEG) and poly (2- (4-vinylbenzyloxy) -N, N-diethylnicotinamide) PDENA-PEG. Hydrotropic agent shows undesired effect and co-absorption, so it was converted into hydrotropic polymer maintaining the hydrotropic properties in the polymeric form. The authors observed that the solubilizing capacity was higher in polymeric form due to high local concentration of hydrotropic moieties in polymeric form. Dialysis method was used and optimized to achieve the highest PTX loading in PDENA-PEG micelles. Polymer with longer PDENA block showed higher loading (37.4%) compared to other systems.

Developed PTX loaded PDENA-PEG micelles were stable up to 30 days and no drug precipitation was observed (21). In another attempt, Park *et al.* (2008) prepared hydrotropic polymeric micelles for oral delivery of PTX. The authors used 4-(2-vinylbenzyloxy) -N, N- (diethylnicotinamide) (VBODENA) as hydrotropic agent and acrylic acid was used for faster release of PTX in SIF (simulated intestinal fluid). The authors prepared five different polymeric micelles varying VBODENA/tBA (t-butyl acrylate) ratio. PTX loading was higher at low pH(<4) in hydrotropic polymeric micelles but as the acrylic acid content increased loading of drug subsequently decreased. They did not find any correlation between pH and acrylic acid content. The best loading in hydrotropic polymeric micelle was observed using dialysis method. The release of PTX from hydrotropic polymeric micelle was higher in SIF than SGF (simulated gastric fluid) (26).

Huh *et al.* (2008) synthesized a hydrotropic block copolymeric micellar system using PEG as hydrophilic block, and poly (4-(2-vinylbenzyloxy-N-picolylnicotinamide)) (P (2-VBOPNA) as hydrotropic block. The authors used atom transfer radical polymerization (ATRP) for the synthesis of both the blocks (Fig. 6). The key factors in hydrotropic effect of PEG-b–P (2-VBOPNA) were pH of the medium, micellization, and block length of P (2-VBOPNA). Micelle formation was observed at higher pH, due to protonation of the PNA group. This protonation of hydrotropic groups, PNA affects both micelle formation and hydrotropic effect. The authors reported that at the lower pH hydrotropic effect was the main mechanism of solubilization. Solubility of PTX was increased up to more than two folds compared to any other conventional method. Release of PTX was slightly dependent on the block length of P(2-VBOPNA). Micelle with relatively shorter hydrotropic block showed slightly faster PTX release (45).

Doxorubicin

Doxorubicin (DOX), chemically a potent anthracycline, is one of the most widely studied anticancer drugs reported to be delivered through different novel carriers such as liposomes, dendrimers and nanoparticles (86–92) including polymeric micelles. DOX has exceptional potential applicability against number of solid tumors as well as AIDS related Kaposi's sarcomas. Conversely, it also poses several adverse effects such as myelosuppression, thrombocytopenia and anemia, etc. These adverse effect(s) necessitates the strong need of innovative delivery approach for the safe and effective delivery of DOX. As mentioned earlier several approaches have been devised and reported in the past, however very few approaches have resulted into commercial reality so far. DOX liposomes (Myocet); PEGylated DOX liposomes (Doxil) are some of the examples which have resulted as an outcome of this research.

Polymeric micelles based delivery is another example of successful approaches for the effective delivery of DOX in the recent years (Fig. 5). Kataoka and coworkers have an extensive research history since late 80s, in DOX delivery using polymeric micelles of PEG-PBLA and other copolymers (9, 38, 93–97). One of the earliest studies reported by Kataoka and coworkers was based on DOX-conjugated poly (ethylene glycol) -poly (α, β-aspartic acid) polymeric micelles (94). In 1990, Yokoyama *et al.* reported DOX conjugation to PEG-poly aspartic acid copolymers. The study reported the various optimization steps followed to conjugate and develop an appropriate ratio of DOX for the development of DOX conjugated PEG-poly aspartic acid micelles. The developed polymeric micelles were found to be stable and highly water soluble. The authors called the conjugated DOX and poly

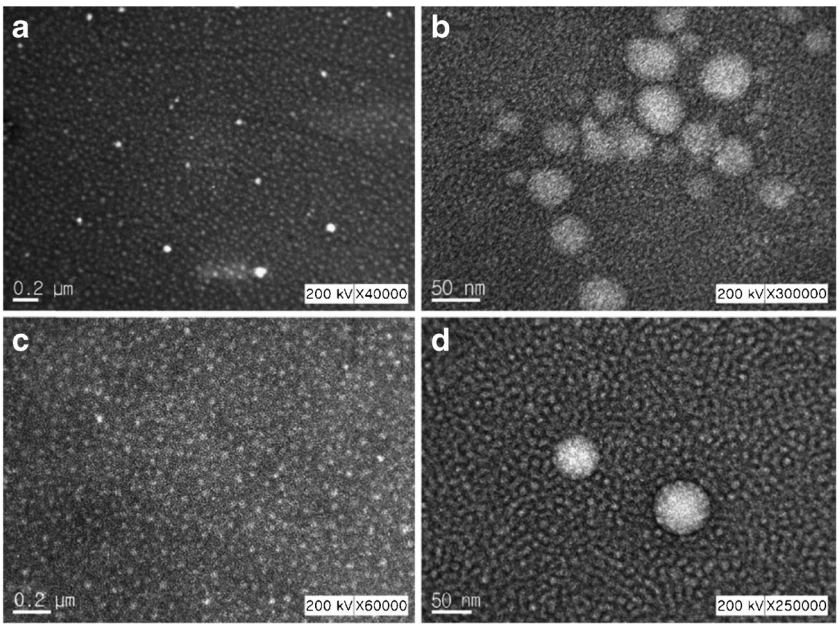

Fig. 6 Transmission electron microscopic (TEM) images of the hydrotropic polymer micelle without (**a**, **b**) and with (**c**, **d**) paclitaxel. (Reprinted with permission from Reference no. (45)).

aspartic portion of copolymers as "polymer drug". *In vivo* anticancer activity of this polymeric drug against P-388 mouse leukemia model showed excellent *in vivo* potential (98). In further studies, the same group of researchers explored *in-vivo* applicability of both DOX encapsulated and conjugated polymeric micelles based on poly(ethylene glycol) -poly (α, β-aspartic acid) micelles (38, 95–97). NK911 is a polymeric micelle based formulation of DOX which has recently been reported to be in clinical trials of Phase II (33) and reflects the possibility of commercialization in this research area.

Initiated in 1997, Kataoka and coworkers reported DOX encapsulated poly (ethylene oxide) -block-poly (b-benzyl-L-aspartate) (PEO-PBLA) block co-polymeric polymeric micelles. The maximum yield of the DOX loading was 65%, while the loading efficiency was found to be 8%. It was observed that most of the drug was loaded in PEO-PBLA copolymeric micelles without any degradation. The loaded polymeric micelles were in the size range <50 nm. Upon release studies in PBS (pH 7.4) in the presence of serum albumin, slow release and possibility of passive targeting to solid tumor was reported (9). Similarly, in the year 2000, Kataoka *et al.* reported pharmaceutical and biological significance of DOX loaded PEG-PBLA [poly (ethylene glycol) –poly (β-benzyl-L-aspartate)] copolymer micelles. Oil in water emulsion method for DOX loading was used and 15–20% drug loading was observed with a size of approximately 50 nm. The authors reported that the DOX developed dimer while loaded in polymeric micelles. A remarkable improvement in blood circulation of DOX using PEG-PBLA micelle was reported probably due to less recognition and uptake from the reticulo-endothelial system. *In vivo* results revealed the higher effectivity of DOX using polymeric micelles (7). In 2005 Kataoka *et al.* reviewed the application of polymeric micelles in drug as well as gene delivery (99).

Nakanishu *et al.* further reported NK911 polymeric micelle for the delivery of DOX. These are the DOX loaded PEG-b-poly (α,β-aspartic acid) polymeric micelles based optimized formulations proceeded for the clinical trials under the name NK911 as mentioned above. The authors developed DOX conjugated as well as DOX loaded PEG/poly aspartic acid polymeric micelles. Conjugated DOX acted as a reservoir of DOX and stabilized the micelles. The developed polymeric micelles were more effective against cell lines, such as P388, Colon 26, M5076, MX-1 and Lu-24. MX-1 and Lu-24, compared to DOX. The AUC of NK911 was 3–4 folds higher than free DOX. In all xenograft models free DOX showed higher toxicity than NK911 and also the accumulation of NK911 was higher in cells due to EPR effect (13). In 2002, Tsukioka *et al.* compared NK911 with Doxil and reported the superior activity and properties of NK911 (100). Doxil was found to deliver more drugs to the tumor site via EPR effect. Matsumura *et al.* (2004) reported phase I clinical trial and pharmacokinetic evaluation of NK 911. MTD for phase II was determined at 50 mg m^{-2} dose every 3 weeks (101). In an another effort to develop biodegradable polymeric micelles for DOX, Park *et al.*(2001) reported micelles composed of poly (ethylene glycol) -poly (D L- Lactic-co-glycolic acid (PEG-PLGA) block copolymers. The DOX was encapsulated and conjugated to PLGA via a carbamate bond, which resulted in its sustained release pattern from the developed PEG-PLGA based micelles. The loading efficiency of DOX reported by the authors was excellent (99.09%). DOX conjugated PEG-PLGA polymeric micelles were found to be ten folds cytotoxic then free DOX against HepG2 cell lines (4).

Further, in 2002, Park *et al.* (2002) conjugated DOX with biodegradable di-block copolymer composed of poly (L-lactic acid) and methoxy-poly (ethylene glycol) (mPEG) via acid labile linkage. There were two acid labile bonds between DOX and PLLA i.e. a hydrazone bond and cis-acotinyl bond. Hydrazone bond was more prone to conjugation efficiency of DOX in PEG-PLLA. DOX release was governed by this acid cleavable bond. DOX release was almost two fold faster at pH 3 than at pH 7. IC_{50} value was reported 5 times lower than free DOX against the human lymphoblast cell line (HSB-2) which might be due to higher uptake of micelle by endocytosis via an active transport mechanism (5).

Lee *et al.* (2003) reported pH sensitive and folate mediated mixed polymeric micelles composed of (polyHis; *M* 5000) / PEG (*M* 2000) and poly (L-lactic acid) (PLLA) (*M* 3000) /PEG (*M* 2000) block copolymers with or without folate conjugation. PolyHis was coupled with PEG through an amide bond and folate was conjugated via an ester bond to this copolymer. pH dependent cytotoxicity in MCF-7 cells was investigated. pH dependent cytotoxicity rely only on release of ADR at different pH. In case of folate conjugated micelles (polyHis/PEG-folate) cell viability was decreased due to higher uptake of micelle through folate receptor and ADR release influenced by extracellular pH. The fusogenic activity of polyHis facilitated ADR delivery, in endosomes. The authors suggested that due to combined pH-triggered release and mechanism, these micelles can be used for the treatment of solid tumors (102). The same group of researchers started to look into the other aspects of polymeric micelles, such as pH sensitive polymeric micelle, Bae *et al.* (2005a) reported these micelles for a resistant MCF-7 tumor, which were surface decorated with folate. The authors used a mixture of two block copolymer (poly-His/PEG-folate) (75 weight %) and poly (l-lactic acid) -b-PEG-folate (PLLA/PEG-folate) (25 weight %) for micelle formation. Four different polymeric micelles PHSM/f (pH sensitive micelle with folate), PHSM (pH sensitive micelle without folate), PHIM/f (pH insensitive micelle with folate) and PHIM (pH insensitive micelle without folate) were prepared. The loading efficiency of all micelles was 75–80%. PHSM/f showed more than 90% cytotoxic activity against DOX resistant MCF-7 cells. The volume of the tumor treated with PHSM/f was 2.7 times smaller than those treated with

free DOX or PHIM/f and 1.9 times smaller than those treated with PHSM (42).

In their further study in 2009, the same group of researchers (11) reported physicochemical aspects of similar kind of DOX loaded pH sensitive polymeric micelles. The authors further used mixture of poly (L-histidine) -b-poly (ethylene glycol) /poly (L-lactide) -b-poly (ethylene glycol) (PH-PEG and PLLA-PEG) (75/25, wt. %) copolymers. Micelles prepared at 4°C were having size of 153±15 nm and DOX loading efficiency of 90±5%. The authors reported that the drug was mainly located in the hydrophobic core as evidenced by various techniques. The slower degradation rate was reported for the encapsulated DOX (11). In the same year, Jeong *et al.* (2009) reported multi block copolymer poly (γ-benzyl l-glutamate)/poly (ethylene oxide) (abbreviated as GEG) based polymeric micelles for the delivery of ADR. Authors synthesized three different polymeric micelles by varying the overall weight ratio (polymer/ADR), GEG-ADR-1, 2, 3. Release in aqueous media was faster for the first day followed by slow release over a period of 1 week. In higher drug content, micelles release was slower than low drug content micelles, due to crystallization of drug at higher concentration. They investigated antitumor activity *in-vitro* in CT26 cells and observed polymeric micelles (GEG-ADR-2) did not significantly affect survivability of the cells due to slow release of ADR (103).

In a recent study, Han *et al.* (2011) reported the role of composite micelles based on PEG (polyethylene glycol) -PCL (polycaprolactone) / Puronic 105 for the delivery of DOX to reverse the drug resistance in the human myelogenous leukemia (K562/ADR) cells. The developed composite micelles were 4 folds cytotoxic than the free drug. The authors synthesized three different types of the polymeric micelles i.e. PEG–PCL micelle, P105 micelle and PEG–PCL/P105 composite micelle. The authors also studied the mechanism by which the MDR in the leukemic cells was reversed and they found that micelles containing P105 significantly decreased the Pgp expression which is one of the mechanisms responsible for the MDR (8).

Yu *et al.* (2014) reported galactosylated cholesterol modified glycol chitosan (Gal-CGCH) micelles. As per the reported theme particle size and aggregation of micelles can be increased by increasing galactose substitution, means lower the galactose smaller the size of particles. They prepared four different polymeric micelles without galactose and with different ratio of galactose. They reported that galactosylated micelle could enhance the cellular uptake of DOX with higher cytotoxic activity against HepG2 cells then free DOX (3).

Hami *et al.* (2014) reported DOX conjugated PLA-PEG-folate based polymeric micelle for tumor targeted delivery. The authors prepared two types of polymeric micelles i.e. folate mediated (targeted) and non-folate mediated (non-targeted). Conjugated micelles were prepared by dialysis method. DOX was conjugated 39.6% (molar percent) via a hydrazone bond. They found the zeta potential was more negative in the case of targeted micelle and this led to enhanced dispersion stability of the micelle. Targeted micelles exhibited higher cytotoxicity against SKOV3 cells than the non-targeted ones with IC_{50} concentration 4.65 and 13.51 μg/ml, respectively. Due to over expression of folate reductase (FRs) on surfaces of SKOV3, uptake of folate mediated micelle was higher and was observed to have 3 folds higher activity. Due to hydrazone bond the release of DOX from micelle was slow and sustained (6).

Kwon *et al.* (1994) investigated entrapment of ADR in AB block copolymer based (poly (ethylene oxide-co-β-benzyl-L-aspartate) (PEO-PBLA)) polymeric micelles using a simple dialysis procedure. The size of the PEO-PBLA polymeric micelle was 30 nm and the drug was entrapped 10% *w*/*w* of PEO-PBLA. The authors concluded that ADR get self- aggregated in the polymeric micelle and was gradually released over a period of time (97). Jeong *et al.* (1997) concluded in their review that an aqueous solution of biodegradable, thermosensitive hydrogel consisting blocks of poly (ethylene oxide) and poly (L-lactic acid) can be used for delivery of the drugs because at 45°C solution can be injected and at body temperature it get solidify and release the drug in sustained manner (104). Yokoyama *et al.* (1998) entrapped ADR by chemical conjugation as well as physical entrapment in polymeric micelle consisting of poly (ethylene glycol) – poly (aspartic acid) block copolymers and observed superior activity (105).

Camptothecin

Camptothecin (CPT), a quinoline alkaloid derived insoluble anticancer drug, is obtained from the bark, wood and fruit of *Camptotheca acuminata*. The chemical structure of the CPT includes a five-ring backbone comprised of a (3, 4- β) quinoline moiety, an indolizinone and a α-hydroxy- δ-lactone ring, with a chiral center at position C_{20}. CPT acts by inhibiting the topoisomerase I activity and leads to the cytotoxic effect in cancerous cells. To improve the CPT solubility in water and to reduce its toxicity, several analogs/derivatives of CPT has been reported such as irinotecan, SN38, topotecan, 10 hydroxycomptothecin, 9 amino campothecin etc. Irinotecan and topotecan has been clinically approved and SN38 represents one of the most active analogues of CPT for anti-tumor activity (106, 107). Researchers have attempted to deliver CPT through various approaches, including polymeric micelles as well (Fig. 5).

Hayama *et al.* (2007) reported modified polymeric micelle for targeted drug delivery against cancer. The authors modified CPT loaded polymeric micelle by folate conjugation. They prepared four different CPT loaded micelles as plain micelles, 0.03F-micelle, 0.1F-micelle and 0.2F-micelle. They

reported, cellular uptake of F-micelle by FR (folate receptor) -mediated endocytosis in KB cells, and reported localization of micelle in endosomal compartment. Cytotoxicity was checked against FR (+) KB cells and FR (−) HepG2 and observed higher toxicity of 0.03F-micelle and 0.2F- micelle than plain micelle in KB cells, while this was not observed in HepG2 cells. They suggested F-micelle can be modified for tumor targeted delivery through folate receptors (28).

Opanasopit *et al.* (2004) designed a block copolymer for passive tumor targeting of CPT. They synthesized poly (ethylene glycol) –poly (β-benzyl L-aspartate) (PEG-PBLA) block copolymers. The PBLA chain was modified by alkaline hydrolysis of its benzyl group followed by esterification with benzyl, *n*-butyl, and lauryl groups. Ten different polymers of varying molecular weights of PEG, the number of aspartate units, esterified group and percent esterification were prepared. These were denoted as 5-27-Bz-75, in which 5 denotes MW of PEG 5000, 27 denotes the number of aspartate units, Bz is benzyl, 75 is percent esterification. They observed block copolymer with high benzyl (esterified group) content showing high loading efficiency. They reported, PEG with high MW and high benzyl residue retard both the drug loading and drug release. High benzyl content leads to lower drug release and PEG with low MW leads to more stable polymeric micelle. 5-27-Bz 69 showed highest loading efficiency. Polymer with high benzyl content showed sustained release. Study concluded that there should be a balance between hydrophobic and hydrophilic content to get stable micelle. The drug content may also affect the loading efficiency and stability of the micelle. Lactone (active form of CPT) can be stored in micelle for a long period with high concentration (29).

Miscellaneous

So many other studies reported the delivery of other anticancer drugs using polymeric micelles (Fig. 5). The approaches thus include multiple or dual drug delivery through micellar delivery. Cho *et al.* (2013) reported combined delivery of PTX, cyclopamine and gossypol (Fig. 5) by polymeric micelles, composed of poly (ethylene glycol) -block-poly (ε-caprolactone) (PEG-b-PCL). The authors prepared different polymeric micelles e.g. 1-drug PEG-b-PCL micelle, 2-drug PEG-b-PCL micelle and 3-drug PEG-b-PCL micelle according to drug incorporated into micelles. 3-drug PEG-b-PCL micelle was found to have highest loading efficiency. *In-vitro* cytotoxicity was investigated against SKOV-3 Luc and ES-2 Luc. 3-drug PEG-b-PCL more effective against ES-2 luc cells. 3-drug PCL-b-PCL showed high cytotoxic activity after intraperitoneal administration (108). Blanco *et al.* (2007) reported poly (ethylene glycol) -block-poly (D, L-lactide) (PEG-PLA) polymeric micelles for β-lapachone (β-lap) delivery. They prepared micelle by three different methods, e.g. dialysis, film sonication and solvent dialysis. They reported micelles prepared by film sonication has highest loading efficiency and higher micelles yield among three methods. Diameter of micelles was slightly higher in case of sonication it might be due to high drug loading. They observed biphasic drug release first 18 h release was faster and after 18 h it was slow. B-lap is bio-activated by NADP (H): quinoneoxide reductase 1 (NQO1), an enzyme overexpressed in a variety of tumors They reported cytotoxic activity of β-lap on DU-145 and MDA-MB-231 cell lines (NQO1+ and NQO1-) and observed β-lap was active against NQO1+ cells and inactive against NQO1- cells. They performed several bio-assays in H596 cells to identify vital component and found NAD loss when cells exposed to micelles at 10 μM dose. No DNA damage was observed in free β-lap exposure to cells. They observed exponential decrease of NAD in cells as the time of exposure is increased (109). Yokoyama *et al.* (1998) reported incorporation KRN5500 (KRN) in polymeric micelles and control particle size. They examined three different block polymers poly-(ethylene glycol) -b-poly (β-benzyl L- aspartate) (PEG-PBLA) and two derivatives of it PEG–P (Asp, BLA) by hydrolysis and PEG–P (C16, BLA) by esterification. They reported for the incorporation of KRN the length of the hydrophobic chain and solvent, which used to play a significant role. They reported if the KRN concentration will increase than a higher association of micelle will be there. PEG-P (C16, −BLA) and DMSO gave a homogenous and high incorporation of KRN using dialysis. They used dialysis for incorporation of KRN, and sonication for reducing the size of polymeric micelles (from associated dispersive micelles to non-associated micelles) and found appropriate micelle (71 nm). They suggested this method can be used for less or hardly soluble drugs (105).

POLYMERIC MICELLES USED IN THE ANTICANCER DRUG DELIVERY

PEG-PLA

PEG has been used as a hydrophilic block of polymeric micelles in most of the reported studies. However the choice of using hydrophobic block (core) varied enormously. Though a variety of block copolymers were used as core, however few polymers, as hydrophobic block, have been used repeatedly, such as PLA and PBLA. In one of such report by Yasugi *et al.* Poly (ethylene glycol) -poly (D, L-lactide) block copolymers (PEG-PLA) were prepared by ring-opening polymerization at room temperature under argon atmosphere. The synthesized PEG-PLA copolymers were used for delivering a hydrophobic drug. PEG acted as its outer palisade because it is hydrophilic, while PLA was inner core, which protects the drug from the outer aqueous medium. The size of the micelles was 50 nm, which helped in achieving high extravasations efficacy based on the EPR effect (110). Riley *et al.* (1999)

reported PEG-PLA based micellar like nanoparticles prepared by the ring opening polymerization of D, L-lactide in the presence of MPEG using stannous octoate as a catalyst. The authors used diblock copolymer because the core-shell type structures are best achieved with amphiphilic AB block copolymers. PEG is usually the hydrophilic buoy chosen to provide a hydrated steric barrier. Following intravenous administration, this PEG corona enabled colloidal carriers, avoids recognition and sequestration by the body's defense system; the reticuloendothelial system (111). Huh *et al.* (2005) prepared PEG-PLA copolymers and reported the hydrotropic polymeric micelles for PTX delivery (112). PEG-PLA was also reported by Blanco *et al.* (2007) for lapachone. The authors prepared PEG-PLA block copolymers by ring opening polymerization method (109). Li *et al.* (2011) prepared PEG-PLA block copolymers by ring-opening polymerization of lactic acid in the presence of PEG macro-initiators. The unusual fusiform micelle morphology implied a different self-assemble behavior for the nonlinear amphiphilic copolymeric system. It is also identified that the unusual fusiform micelles have significantly higher drug loading capacity and encapsulation efficiency than those formed by linear MPEG-PLA with similar molecular weight (23). Hami *et al.* (2014) conjugated the PEG-PLA polymeric micelles with folate as a targeting moiety for tumor targeted delivery of DOX. They prepared PEG-PLA block copolymer by ring opening polymerization of lactide in the presence of carboxylic acid. Briefly, vacuum-dried lactide and carboxylated PEG were allowed to react in anhydrous toluene in the presence of and tin (II) 2-ethylhexanoate as a catalyst at the refluxing temperature of toluene. The PLA-PEG copolymer was extracted with chloroform after evaporation of the reaction solvent. Folic acid has a small size, non-immunogenicity, low molecular weight and higher stability. Therefore, micellar delivery systems have further been modified with target-specific ligand i.e. folate to enhance tumor specificity and improve the tumor uptake by folate receptor-mediated endocytosis (6).

PEG-PLLA

Similar to PEG-PLA, poly (L-lactic acid) -b-poly (ethylene glycol) based polymeric micelles has been reported equally in the literature for the delivery of anticancer and other drugs. Zhang *et al.* (1996) reported polymeric micelles consisting of MePEG-PDLLA (poly D L-lactide-co-methoxy polyethylene glycol) di-block copolymer prepared by the ring opening polymerization procedure (61). Yoo *et al.* (2002) also reported PEG-PLLA diblock co-polymeric micelles with the same procedure. The authors synthesized PEG-PLLA di-block using L-lactide and mPEG with heating under a nitrogen atmosphere and in the presence of stannous octoate and was maintained for 6 h. The authors conjugated DOX to these copolymers to achieve sustained release (113). MePEG-PDLLA based polymeric micelles were also reported by Burt and Liggins (2002). Diblock copolymer in the reported study was prepared using MePEG and PDLLA by ring opening of PDLLA monomer. The developed diblock copolymer as micelle enhanced the solubility of a hydrophobic drug by 5000 folds. Additionally, MePEG-PDLLA micelles have been shown to be biocompatible and non-toxic in a range of *in vitro* and *in vivo* evaluations (67). Similarly, Kim *et al.* reported the polymeric micelles based on PEG-PLLA using ring opening polymerization using stannous octoate as catalyst (27). In an excellent study by Yin and Bae in 2009, pH-sensitive micelles made from a mixture of poly (L-histidine) -b-poly (ethylene glycol) /poly(L-lactide) -b-poly(ethylene glycol) were reported. PEG-PLLA micelles were very stable above pH 7.4 but destabilized as the pH decreased below 6.8, which was attributed to increased electrostatic repulsions arising from the progressive protonation of the imidazole rings on the poly (L-histidine) blocks. Poly (l-histidine) (polyHis) is known to have an endosomal membrane disruption activity induced by a "proton sponge" mechanism of the imidazole groups. Thus, when the polymeric miceller system with the polyHis core was employed, it became in a more effective mode of cytosolic delivery of anticancer drug (11).

PEG-PBLA

Poly (b-benzyl-L-aspartate) is another polymeric block which has been used extensively for the synthesis of AB block copolymer based polymeric micelles. Yokoyama *et al.* (1998) reported polymeric micelle forming of the PEG - PBLA. The authors prepared block copolymer by polymerization of b-benzyl L-aspartate *N*-carboxy anhydride initiated by a terminal amino group of α-methoxy-ω-aminopoly (ethylene glycol) (105). Kataoka *et al.* (2000) prepared PEG-PBLA polymeric micelles through ring opening polymerization of b-benzyl-L-aspartate. *N*-carboxyanhydride was initiated from the terminal primary amino group of a-methoxy-v-amino poly (ethylene glycol) in the presence of Nippon oil and fats under an argon atmosphere in chloroform. Dimer derivatives of DOX as well as DOX itself were revealed to be entrapped in the micelle, the former seems to improve micelle stability due to its low water solubility and possible interaction with benzyl residues of PBLA segments through π–p stacking. A remarkable improvement in blood circulation of DOX was achieved by the use of PEG–PBLA micelles as a carrier presumably due to the reduced RES uptake of the micelles through a steric stabilization mechanism (7). Opanasopit *et al.* (2004) also reported polymeric micelles consisting of PEG-PBLA block copolymer and found promising results in delivering camptothecin. PBLA has been used in several other reports as a hydrophobic block of copolymers, as mentioned in subsequent paragraphs (29).

Miscellaneous

In the initial years of polymeric micellar research, scientists used polyethylene oxide (PEO) and other polymers as corona instead of PEG, as mentioned above. Micelles of block copolymers poly(ethylene oxide)-*block*-poly(b-benzyl-L-aspartate) (PEO-PBLA) were reported by Kataoka *et al.* (1997) in which shell consisted of a hydrophilic PEO block and core consist hydrophobic block PBLA. The shell was again believed to interact with biological milieu and affects pharmacokinetics and disposition. There were evidences for prolonged circulation times of block copolymeric self-assembled micelles. The authors used o/w emulsion method for loading of the drug and copolymer stabilized the emulsion. The authors reported chemical stability of DOX loaded in PEO-PBLA micelles (9). Self-assembled poly(ethylene oxide) -block-poly(L-amino acid) (PEO-b-PLAA) were reported by Lavasanifar *et al.*(2002). Chemical modification of the core-forming PLAA block was used to adjust and optimize the properties of PEO-*b*-PLAA micelles for drug delivery. Micelle-forming block copolymer–drug conjugates, micellar nanocontainers and polyion complex micelles have been obtained that mimic functional aspects of biological carriers, namely, lipoproteins and viruses. PEO-*b*-PLAA micelles may be advantageous in terms of safety, stability, and scale-up. They used PEO-b-PLAA because poly(ethylene oxide) -*b*-poly(L-amino acid) (PEO-*b*-PLAA) as synthetic analogs of natural carriers with a unique ability for chemical modification. Free functional groups on a PLAA block provide sites for the attachment of drugs, drug compatible moieties or charged therapeutics such as DNA. In either case, it may be possible to fine tune the structure of the core-forming block and enhance the properties of PEO-*b*-PLAA micelles for drug delivery. Folate was used as targeting moiety which gets associated with folate receptor (114). Folate linked PEG-distearoylphosphatidylethanolamine (Folate-PEG_{5000}-DSPE) micelles were reported by Hayama *et al.*(2007). The novel lipid-based modification method to polymeric micelles is applicable to antibody, peptides, or other ligands (28).

Huh *et al.*(2008) reported polymeric micelles for aqueous solubilization of PTX consisting of PEG as hydrophilic block and poly (4-(2-vinylbenzoxy-N-picolylnicotinamide)) (P (2-VBOPNA)) as a hydrotropic block. Hydrotropic block did not form micelles at pH 2 or below, due to protonation of PNA group, but solubility was increased due to hydrotropic activity. At higher pH solubility increased due to deprotonation of P (2-VBOPNA) leading to micelle formation (45). PTX was also delivered using novel self-assembling poly (ethylene glycol)$_{750}$-block-poly (ε-caprolactone-co-trimethylene carbonate) (PEG-p-(CL-co-TMC)) polymeric micelles by Preat *et al.* (2009) to solubilize PTX without Cremophor EL and to be used as a safe and effective delivery system for PTX. The major advantages of PEG-p-(CL-co-TMC) micelles is self-assembling upon gentle mixing with water and that too not organic solvent neither dialysis or evaporation step is required for encapsulation (14). Jeong *et al.* (2009) used multiblock copolymers poly (γ-benzyl L-glutamate) (PBLG) and PEO to prepare polymeric micelles (103). Lee *et al.* (2003) reported pH sensitive mixed polymeric micelles composed of poly (L-histidine) (polyHis; *M* 5000) /PEG (*M* 2000) and poly (L-lactic acid) (PLLA) (*M* 3000) /PEG (*M* 2000) block copolymers with folate conjugation (102).

Morreton *et al.* (2010) reported flower-like micelles of poly (ε-caprolactone) -b-poly(ethylene glycol) -poly (ε-caprolactone) (PCL-PEG-PCL) block copolymers. PCL-PEG-PCL triblocks were synthesized by a ring opening polymerization reaction catalyzed by stannous octoate and PCL/PEG hydrophobic/hydrophilic balances were synthesized by a fast and high-yield Microwave-Assisted Polymer Synthesis (MAPS) technique (115). In another study composite polymeric micelles consisting of polyethylene glycol (PEG) –polycaprolactone (PCL) /Pluronic P105 were reported by Han *et al.* DOX was loaded in micelles and proved to inhibit the drug resistance of human myelogenous leukemia (K562/ADR) cells (8).

In an effort, novel polydepsipeptide contained, tri-block copolymer poly(ethylene glycol) -poly-(L-lactide) -poly(3 (S) -methyl-morpholine-2-5-dione) (mPEG–PLLA–PMMD) as self-assembly micelle delivery system for paclitaxel was developed. Tri-block copolymer has more benefits than di-block copolymer such as low CMC value, positive shifted zeta potential and better drug loading efficiency and stability. Among tri-block copolymers PEG-PLLA-PMMD has low cytotoxicity and promotes the anticancer activity of PTX on A-549 and HCT-116 cells (15). Polysorbate 80 coated poly (ε-caprolactone) –poly (ethylene glycol) -poly (ε-caprolactone) (PCEC) micelles were prepared for PTX delivery by Quin *et al.*(2013). Polysorbate 80 altered the bio-distribution pattern and increased PTX concentration in the brain as it has the ability to cross the BBB (19). In another study, galactosylated cholesterol modified-glycol chitosan (Gal-CHGC) micelles were reported by Yu *et al.* (2014). Glycol chitosan was used as hydrophilic segment because of its biocompatibility and excellent solubility in water at all pH. DOX was loaded and prolonged action was reported compared to free drug. Galactosylated micelles could enhance the uptake of DOX into HepG2 cells. Moreover, the cytotoxicity of DOX-loaded galactosylated micelles against HepG2 cells significantly improved in contrast with free DOX and DOX-loaded micelles without galactosylation. These results suggested that Gal-CHGC micelles could be a potential carrier for hepatoma-targeting drug delivery. Galactose moiety was used as a targeting moiety which recognized by an asialoglycoprotein receptor (ASGPR) in liver (3). PEG-b-PLGA copolymeric micelles were reported by Zhang *et al.* (2014). Micelles were loaded with hydrophobic docetaxel (Fig. 5) and combined with chloroquine as an autophagy inhibitor agent to prevent intracellular autophagy which leads to deterioration of their advantages for efficient drug delivery (116).

In general, the researchers have used various combinations of hydrophilic and hydrophobic blocks for the preparation and characterization of polymeric micelles. Some of the combinations as mentioned need further exploration in future to be commercialized based on their promising results *in vitro* and *in vivo*.

CLINICAL OUTCOME, CONCLUSION AND FUTURE PROSPECTS

Nanotechnology has impacted extraordinarily to solve the hurdles of medical research in the last few decades. The use of nano-carriers such as nanoparticles, liposomes, dendrimers, polymeric micelles carbon nanotubes etc. for the treatment of complex disorders and diseases such as cancer, autoimmune disorders etc. have initiated a new era of research. The use of the etiological basis of certain diseases such as cancers (through the EPR effect) has been used as a targeting/delivery strategy of drugs at a specific site. Though a considerable research has been devoted to nanoparticles, liposomes, solid lipid nanoparticles and dendrimers etc. in the past, certain other nanocarriers such as carbon nanotubes, fullerens, polymeric micelles etc. still have unexplored arenas to be worked upon. Polymeric micelles in this regard have an excellent research track record as per as the clinical outcome is concerned. More than 6–7 polymeric micelles based products are in the final stages of commercialization from different labs (Table II). The unique opportunities offered by the polymeric micelles with wide choices of hydrophilic corona and hydrophobic core, makes these nano-carriers, excellent and unique for drug delivery and targeting especially for anticancer drugs. As mentioned earlier, several anticancer drug loaded polymeric micelles are under preclinical investigation now-a-days for improved cancer therapy with enhanced efficacy as well as targeted delivery to the tumors. Genexol-PM (consist of a block copolymer of PEG and poly(D, L-lactide)), a cremophor free PTX loaded polymeric micelle has been evaluated in phase I clinical trials against refractory malignancies and the MTD was found to be 300 mg/m^2 for Phase II (117). Phase II study of Genexol-PM plus cisplatin (Fig. 5) was designed to evaluate safety and efficacy against advanced non-small-cell lung cancer (NSCLC) and was found to be offering significant antitumor activity (118). More recently, Genexol-PM and gemcitabine was also evaluated single arm, single center phase II for evaluation of safety and efficacy as well against NSCLC and has resulted in favorable antitumor activity (119). Single arm multicenter phase II was also designed for Genexol-PM to evaluate safety and efficacy (125). NK 105 (consisting of a block copolymer of PEG and poly (aspartate)), a polymeric micelle for delivery of PTX was evaluated in pre-clinical studies (60) and phase I to evaluate MTD, dose limited toxicities and recommended dose for phase II. Recommended dose was found to be 150 mgm^{-2} for phase II (120). NK 012 (consist of a block copolymer of PEG and poly glutamate (PGlu)), an SN38 loaded polymeric micelle constructed in aqueous medium of self-assembling of block copolymer and its antitumor activity was evaluated against several tumor models including renal cancer, stomach cancer and pancreatic cancer. Two independent phase I clinical trials has been conducted in Japan and USA and observed that NK012 has significant antitumor activity with no intestinal toxicity and phase II studies are going on (121). Another PM based product NC6004 (consist of PEG and a poly(γ-benzyl L-glutamate), a micellar construction for PTX delivery and its phase I and II studies were performed to evaluate safety and efficacy as well (60, 122). SP1049C (consist of pluronic L61 and F127), is under clinical phase III studies (124) while another product NC6300 (consist of a block copolymer of PEG-poly (aspartate)), a polymeric micelle for PTX is also under phase I clinical trial (123). As per the above mentioned details it can be concluded that till date there are few commercial products which are available or yet to be available in the market solely based on polymeric micelles (126). NK105 which is a polymeric micelle based formulation of PTX based on modified mPEG-poly (aspartic acid) copolymer developed by Nippon Kayaku Co. Ltd., for the treatment of recurrent or meta-static breast cancer (127). Similarly, NK012, which is also a miceller product of Nippon Kayaku Co. Ltd. is recommended and used for the treatment of triple negative breast cancer (121). Both of the above products are in the phase III and II clinical trial, respectively. Another commercial product based on polymeric micelles is NC-

Table II Clinical Output of Polymeric Micelles

Polymeric micelle	Block copolymer	Bioactive	Cancer	Clinical phase	References
Genexol-PM	PEG-P(D,L-lactide)	PTX	NSCLC	II	(117–119)
NK105	PEG-P(aspartate)	PTX	Advance stomach cancer	II	(60, 120)
NK012	PEG-PGlu	SN38	Renal, stomach and Pancreatic cancer	I/II	(121)
NK 6004	PEG-PGlu	Cisplatin	Solid tumors	I/II	(60, 122)
NC6300	PEG-P(Asp)	Cisplatin	Liver cancer	I	(123)
SP1049C	Pluronic L61 and F127	DOX	Adenocarcinoma of oesophagus, gastroesophageal junction and stomach	III	(124)

6004 based on mPEG-poly (glutamic acid) having cisplatin as a core drug. NC-6004 has been developed by Nano Carrier Co. Ltd for the treatment of locally advanced or metastatic pancreatic cancer (128).

Compared to conventional dosage forms such as tablets, capsules, emulsions etc. novel drug delivery systems offers several advantages in terms of efficacy, release pattern, safety and patient compliance. Polymeric micelles in particular offers the choice of developing different co-polymeric structure based on the exact formulation requirement. The excellent solution behavior and established characterization methodologies further attracts the researchers to work upon and explore these carriers. Some of the areas such as the delivery of polymeric micelles through alternative route of administration as well as gene delivery and targeting still needs the attention and exploration by researchers in future studies.

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