EXPERT OPINION

Amphiphilic polymeric micelles as the nanocarrier for peroral delivery of poorly soluble anticancer drugs

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Introduction: Many amphiphilic copolymers have recently been synthesized as novel promising micellar carriers for the delivery of poorly water-soluble anticancer drugs. Studies on the formulation and oral delivery of such micelles have demonstrated their efficacy in enhancing drug uptake and absorption, and exhibit prolonged circulation time *in vitro* and *in vivo*.
Areas covered: In this review, literature on hydrophobic modifications of several hydrophilic polymers, including polyethylene glycol, chitosan, hyaluronic acid, pluronic and tocopheryl polyethylene glycol succinate, is summarized. Parameters influencing the properties of polymeric micelles for oral chemotherapy are discussed and strategies to overcome main barriers for polymeric micelles peroral absorption are proposed.
Expert opinion: During the design of polymeric micelles for peroral chemotherapy, selecting or synthesizing copolymers with good compatibility with the drug is an effective strategy to increase drug loading and encapsulation efficiency. Stability of the micelles can be improved in different ways. It is recommended to take permeability, mucoadhesion, sustained release, and P-glycoprotein inhibition into consideration during copolymer preparation or to consider adding some excipients in the formulation. Furthermore, both the copolymer structure and drug loading methods should be controlled in order to get micelles with appropriate particle size for better absorption.

Keywords: anticancer drug, nanocarriers, oral delivery, polymeric micelles, poorly soluble

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1. Introduction

Nowadays, more than half of the people diagnosed with cancer receive chemotherapy. However, most classic chemotherapeutic drugs have poor solubility in water, lack selectivity upon administration, and can easily gain multidrug resistance (MDR). Therefore, it is absolutely essential to design appropriate drug delivery systems for anticancer drugs to achieve improved bioavailability and antitumor efficacy.

During the last decade, many studies have focused on the intravenous administration of anticancer drugs [1]. However, intravenous administration results in significant concentration of toxic drugs in the blood stream immediately after administration and low drug concentration below the desired threshold concentrations toward the end of the dosing interval. Moreover, for intravenous administration, aseptic production and strict material type and particle size control are required which increase the cost for industrial manufacture and limite the application of other promising materials. In contrast, drug delivery via oral route is the most convenient and preferred especially when long-term or daily use is required

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Article highlights.

- Various nanotechnologies have been used to improve the absorption of anticancer drugs. Among them polymeric micelle is one of the most promising one, showing great potential for oral chemotherapy.
- Physicochemical properties of polymeric micelles, such as particle size, charge density, drug loading and stability, can be controlled by adjusting preparing conditions including the ratio of hydrophilic/hydrophobic part, concentration of the copolymer, ratio of drug/polymer, and drug loading method.
- The development of polymeric micelles for peroral chemotherapy is dampened by mainly three limitations, including low drug loading and encapsulation efficiency, poor stability and barrier to transport through the GI membrane.
- Polymeric micelles with specific properties, such as mucoadhesion, permeation enhancing and reducing multidrug efflux, can be obtained by appropriate polymer modification. These polymeric micelles showed encouraging *in vitro* and *in vivo* results.
- According to a limited number of studies on *in vivo* behavior of polymeric micelles after oral administration, polymeric micelles can pass through the GI membrane and show a sustained release profile *in vivo*. Further investigation should be carried out to demonstrate the feasibility of using polymeric micelles for peroral delivery of anticancer drug with sustained release properties.

This box summarizes key points contained in the article.

and it is cost-effective. The feasibility for oral chemotherapy has been demonstrated [2].

Polymeric nanoparticles present a number of advantages to promote oral chemotherapy of these drugs [2], including the ability to encapsulate hydrophobic drugs into their cores preventing direct contact of the drug with the intestinal membrane and to be uptaken by cells directly. Many studies have proven that nanoparticles can be transported through the intestinal membrane through paracellular or transcellular routes [3]. In addition, studies using fluorescence resonance energy transfer (FRET) technique indicate that nanoparticles can pass intestinal barrier while maintaining their integrity [4]. In this way many advantages of nanoparticles for intravenous administration, such as enhanced permeability and retention (EPR) effect, can also be obtained by oral administration. Currently, a number of nanoparticles are available for oral chemotherapy including liposome [5], nanosuspension [6], nanoemulsion [7], nanocrystal [8], and many other nanoparticles [9]. Among them, polymeric micelle is one of the most promising ones which can be formed by self-assembly without the use of oils or organic solvent [2]. They physically entrap poorly water-soluble pharmaceuticals and deliver them to the desired site of action at the concentration exceeding their intrinsic water solubility and thus increase their bioavailability. In addition, the stability of the drug is increased after incorporation by micelles. At present, polymeric micelles have already been used for chemotherapy and a couple of micellar anticancer drug formulations, such as NK911 and Genexol-PM. Those formulations have already advanced to clinical trials for intravenous administration [10,11]. Micelles formed by newly synthesized polymers such as N-octyl-O-sulfate chitosan [12] and poly(lactide)-Vitamin E TPGS [13] have been developed in order to deliver anticancer drugs more effectively via the oral route. Based on the advantages of nanoparticles for oral chemotherapy and successful application of polymeric micelles in anticancer drug delivery, it is reasonable to believe that polymeric micelles would be a promising drug carrier for peroral delivery of anticancer drugs after being appropriately designed.

Therefore, in this review, keeping oral chemotherapy in mind, modifications of several typical hydrophilic polymers with special properties (such as mucoadhesion, permeation enhancing, and avoidance of MDR) for preparing self-assembled micelles are summarized and advantages of diverse modifications are analyzed. Factors influencing properties of the polymeric micelles are scrutinized and challenges of polymeric micelles for peroral delivery of anticancer drugs are discussed.

2. Chemical composition of self-assembled micelles

2.1 Polyethylene glycol (PEG)-based modification

PEG-modified copolymers have been widely used to prepare self-assembled micelles for anticancer drugs [14] with the advantages of reducing the rapid renal clearance of small molecular drugs and evading removal from the plasma by enzymes and the reticuloendothelial system after intravenous administration [15].

However, PEG-based micelles did not show obvious benefit for the delivery of poor soluble drugs in the GI tract [16]. This can be explained by the structure of PEG which is transient, flexible, and has the ability to rapidly change. PEG chains form a polymeric "cloud shell" over the micelle surface with a density high enough to prevent interaction with the intestinal membrane cells and thus cause poor absorption [17]. Fortunately, recent studies have indicated that PEG-based micelles show different properties such as particle size, charge and density of the "cloud shell" by varying molecular weight, chain length and composition of PEG chain; and thus by adjusting these parameters, the absorption of PEG-based micelles in the GI tract can be improved [18,19]. It has been shown that the design of short PEG-based micelles for oral anticancer drug delivery, the extent of transepithelial transport, uptake, as well as the mechanism of transport can be controlled by a judicious choice of density, molecular weight of PEG, and degree of PEGylation. For instance, a PEG with molecular weight of 750 was widely used to prepare self-assembled micelles due to its low cloud point compared to longer PEG chains [20,21]. PEG(750)-p(CL-co-TMC) [methoxypoly(ethylene glycol)-poly(caprolactone/trimethylene carbonate)] polymeric micelles remarkably increased the solubility of risperidone [19]. An

in vivo study indicated that polymeric micelles had a similar bioavailability, a 40% decrease of C_{max} and a 1.7-fold increase of $T_{1/2}$ compared to those of risperidone solubilized in tartaric acid, suggesting that PEG-based micelles could provide a promising opportunity for peroral delivery of anticancer drugs with prolonged effect [19]. In addition, there are studies focused on chemically modifying PEG and mixing PEG with other polymers to overcome the PEG steric barrier to transport through intestinal membrane [22,23]. Yao *et al.* used poly(ethylene glycol-2000)-grafted distearoyl phosphosphatidylethanolamine (PEG_{2000}-DSPE) mixed with TPGS to form micelles as carriers for paclitaxel. The antitumor efficacy result showed that paclitaxel nanomicelles had remarkable antitumor efficacy in inhibiting the tumor volume either by intravenous injection (83.7% at day 35) or by oral administration (75.2% at day 35) [4]. It was reported recently that PEG5000-modified poly (lactide) micelle was an effective nanocarrier for solubilization of poorly soluble cyclosporine and improved oral absorption of the drug [24]. Therefore, further investigation into the influence of PEG molecular weight, density of "cloud shell" and on the transport of its modified polymeric micelles through intestinal membrane is essential.

2.2 Chitosan-based modification

Chitosan is a suitable candidate for peroral drug delivery due to its biocompatibility, biodegradability, permeation enhancing effect, and mucoadhesion [25]. Due to the properties of chitosan, micelles based on chitosan can protect anticancer drugs from degradation in the GI tract and improve drug absorption.

Chitosan itself has a certain antitumor activity and its positive charge can neutralize the negative charge on the tumor cell surface, resulting in selective absorption [26]. Moreover, since chitosan is a hydrophilic and cationic polysaccharide with high chemical reactivity, chitosan derivatives can be readily obtained by chemically attaching varied hydrophobic moieties to the backbone of chitosan [27]. Thus far, some chitosan-based micellar systems for peroral drug delivery have been developed and modifications of chitosan are designed for different purposes as summarized in **Table 1**. As illustrated in the table, chitosan-based micelles show many desirable properties which can lead to a promising peroral chemotherapy: i) increasing the solubility of anticancer drug; ii) prolonging circulation time: According to those studies, the circulation time of micelles *in vitro* or *in vivo* ranges from 1 to 3 days; iii) enhancing the transport through epithelium cell membrane; iv) improving the stability of drug in GI tract; v) bypassing MDR. Based on the studies so far, chitosan shows tremendous potential for peroral chemotherapy. Although uptake tests on Caco-2 cells have showed significantly improved absorption of chitosan-based micelles [28], the *in vivo* transport through the GI tract in a complicated bioenvironment and further into tumor cells are still big challenges. Mo *et al.* developed N-octyl-O-sulfate chitosan for oral delivery of paclitaxel (PTX) [12]. Mucoadhesion and permeation enhancing effects of the copolymer provided a sixfold improvement in the oral bioavailability of PTX compared to that of commercially available product (Taxol) *in vivo* [12]. Further investigation should focus on preparing multifunctional chitosan-based micelles and should provide *in vivo* studies for oral administration nicluding both pharmacokinetics and antitumor efficacy.

2.3 Hyaluronic acid (HA)-based modification

HA is abundant in extracellular matrix (ECM) and synovial fluid [29]. The strong correlation between HA and cell migration or proliferation endues HA with natural biocompatibility and biodegradability [30].

HA can specifically bind to various cancer cells that overexpress cluster determinant 44 (CD_{44}) [31], a cell adhesion protein family frequently and homogeneously expressed in various human cancers [32,33]. The unique biological properties of HA also have highlighted it as a potential targeted macromolecular carrier of antitumor drugs.

Polymeric micelles formed by hydrophobic-modified HA derivatives can first attach to mucosa in the GI tract due to its mucoadhesive property. Subsequently, oligosaccharide HA is able to mediate intracellular transport of those micelles into the blood stream [32]. Due to its various biological functions and excellent physicochemical properties, HA-based micelles have been considered as a promising carrier for peroral drug delivery [34]. Choi *et al.* prepared hyaluronic acid micelles formed by amphiphilic HA conjugates chemically conjugated with various hydrophobic moieties on HA backbone such as 5β-cholanic acid, PLA, and PEG [35]. They controlled the degree of substitution (DS) by varying the feed ratio of hydrophobic parts to HA. The results showed that the DS of copolymer significantly affected the particle size and the *in vivo* biodistribution in tumor-bearing mice [35]. Receptor-mediated tumor targeting behavior of HA micelles was evaluated *in vivo* by competitive inhibition with free HA. The study demonstrated that HA micelles selectively targeted to tumor tissue, due to both passive accumulation via the EPR effect and active targeting by the receptor-binding affinity of HA to CD_{44} with high specificity *in vivo* [36], indicating that self-assembled HA-based micelles can be potential peroral drug carriers for cancer therapy. In addition, HA was also conjugated directly with various chemical drugs, such as PTX, doxorubicin, 5-fluorouracil, butyric acid, and mitomycin C [37,38]. The results showed significant enhancement of the absorption and the antitumor effect of the drugs both *in vitro* and *in vivo*.

Although HA has a great potential for peroral delivery [39], its application in micellar technology is limited in three respects. First, to achieve the special biological properties of HA, HA should be controlled to have diverse molecular weights (MW) and a narrow MW distribution with a well-defined specification. Second, both high molecular mass and poor liposolubility of HA inhibit the gastrointestinal absorption [39]. Thirdly, concerning the fast degradation of HA *in vivo*, the release period of HA delivery system should be long enough to compete with

Table 1. Chitosan-based polymeric micelles for drug peroral delivery.

Material	Model drug	Modification purpose	*In vitro* and *In vivo* study	Prepare method	Ref.
Tocopherol succinate-chitosan	Ketoconazole Itraconazole	Inhibiting P-gp Increasing the solubility of model drug	Uptake test of ketoconazole on Caco-2 cell: 3.4-fold increase of apparent permeation	Solvent evaporation method	[97]
N-deoxycholic acid-chitosan	Paclitaxel	Emulsifying fat in the intestine Increasing the solubility of model drug	*In vivo* test: bioavailability is threefold compared with that of an orally dosed Taxol	Solvent evaporation method	[98]
Stearic acid-chitosan	Doxorubicin	Prolonging drug release	Transport mechanism on Caco-2 cells: mainly pinocytosis and partially paracellular route. Pharmacokinetic: 25% absolute bioavailability	Dialysis	[75]
Octyl -chitosan	Etoposide	Significantly increasing the solubility of Paclitaxel inhibiting P-gp	Pharmacokinetic study by oral administration: AUC increased by sixfold compared to Taxol; Uptake study: drug uptake by Caco-2 is 9.88-fold higher than that of Taxol. Transport across Caco-2 cell: Papp increased from 0.47 to 1.22 compared to etoposide control due to P-gp inhibition		[28]
Chitosan	Docetaxel	Increasing solubility of model drug by chemical conjugation Inhibiting P-gp	Pharmacokinetic: AUC is four times higher than that of DTX (i.v.) Antitumor efficacy: 48% tumor inhibition compared to 50% tumor inhibition in DTX (i.v.)	Chemical conjugation	[96]

Table 2. Mixed pluronic copolymers for drug delivery.

Pluronic	Mixed polymer	Model drug	*In vitro* studies	Ref.
Pluronic L61, L62, P85	MPEG-PLA	Docetaxel	3 days released 36 – 67%; cellular uptake: inhibiting P-gp function	[46]
P 105	D-α-tocopheryl polyethylene glycol 1000 succinate	Camptothecin	72 h released 70 – 80%	[56]
F127	Tween 80	Docetaxel	175 h released 92%; cytotoxicity on MCF-7 cells and SKOV-3 cells: DTX mixed micelles > DTX PF127 micelles > free DTX	[47]
F127	P123	Paclitaxel	2 h released 33.4 – 46.3%	[99]

other long-acting systems [40]. By overcoming those limitations, oral chemotherapy using HA-based micellar system will be more promising. The following strategies can be taken into consideration. For example, the molecular mass of HA should be controlled and further hydrophobic modification of HA using the experience from chitosan modifications should be carried out in order to improve oral absorption, drug release, and active tumor-targeting function.

2.4 Pluronic-based modification

Pluronic block copolymers with different hydrophilic ethylene oxide units and hydrophobic propylene oxide units are characterized by a different hydrophile–lipophile balance (HLB) [41]. The micellization of pluronic is initiated by increasing the temperature up to its critical micelle temperature (CMT) or at the concentrations above the critical micelle concentration (CMC) [42]. The micelles consist of a core and a corona. The core is formed of poly(propylene oxide) (PPO) (hydrophobic group) and the corona of hydrated poly(ethylene oxide) (PEO). Pluronic copolymers are highly surface-active and form micelles within a well-defined range of concentrations and temperatures, which depends on the molecular architecture of the pluronic component. In addition to their ability to self-assemble into micelles, some pluronic block copolymers are shown to be potent biological response modifiers capable of sensitizing multidrug-resistant (MDR) cancer cells and enhancing drug transport across cellular barriers, such as polarizing intestinal epithelial cells, Caco-2 cells, and

Table 3. TPGS application for drug delivery.

Polymer	Model drug	*In vitro* and *In vivo* studies	Ref.
Pluronic F127/TPGS	HCPT	20 h release 80%	[100]
Pluronic P105/TPGS	CPT	The cytotoxicity on MCF-7 cells: P105/TPGS micelles > P105 micelles	[56]
Poly(ethylene glycol)2000-phosphatidyl ethanolamine/TPGS	Paclitaxel	28 h release 20%; The cytotoxicity on Caco-2: drug loaded micelles (0.2 – 20%) > empty micelles (95.7 – 113.5%)	[53]
	CPT	72 h release 20 – 25%; The cytotoxicity on MCF-7, LLC1 and HT29 cells: PEG-PE/TPGS micelles > free CPT	[101]
PLGA/TPGS	Paclitaxel	One month release 5 – 30%	[50]
PLA-TPGS	Paclitaxel	PBS (pH 1.6 and 6.8) 30 days release 51 – 80.3%; cellular uptake: PLA-TPGS micelle increase 1.2- to 1.4-fold compare to PLGA nanoparticles; The viability on Caco-2 cells: PLA-TPGS micelles (58.9%) < free Paclitaxel (61.4%)	[13]

brain endothelium [43]. Moreover, the strong mucoadhesive property of pluronic copolymers endues the micelles with the ability to interpenetrate into and anchor on the mucosa [44]. Based on all those desirable properties, pluronic micelles are considered as a potential candidate for oral delivery of drugs and tumor-specific delivery of anticancer agents. Suk *et al.* prepared drug-loaded pluronic micelles to get through the intestinal barrier, resulting in improved oral bioavailability [45]. To obtain synergistic properties, mixed pluronic micelles and modified pluronic micelles are usually prepared which are summarized in **Table 2**. As shown in **Table 2**, pluronic micelles provide a prolonged circulation time of up to 3 days and exhibit significant inhibition of P-gp [46,47].

2.5 Tocopheryl polyethylene glycol succinate (TPGS)-based modification

D-α-tocopheryl polyethylene glycol 1000 succinate (Vitamin E TPGS or simply TPGS) is an amphiphilic compound consisting of a lipophilic alkyl tail and a hydrophilic polar head portion [48,49]. When mixed with other polymers, TPGS can increase drug loading using the drug as the core component which can directly improve drug incorporation [50,51].

TPGS has many advantages as a potential carrier for oral delivery. TPGS can inhibit both pre-systemic drug metabolism and intestinal efflux mediated by P-gp[102], therefore increasing the oral absorption of anticancer drugs [13,48]. The mechanism of TPGS inhibiting P-gp-mediated efflux is that the interaction with the lipid bilayer leads to increased membrane permeability and thus enhances the trans-membrane diffusion of P-gp substrates. This process includes competitive inhibition of substrate binding, alteration of membrane fluidity, and inhibition of the efflux pump ATPase activity [48,52]. Rupa *et al.* demonstrated the uptake of RH-123-loaded micelles formed by PEG-PE and TPGS was P-gp-independent in Caco-2 cells [53]. This result proved that the administration of P-gp substrate (RH-123 or an antitumor drug) in TPGS-based micelles can reduce the efflux mediated by P-gp in Caco-2 cells.

Many other studies [13,53,54] have also demonstrated that TPGS can be used as a suitable carrier for oral delivery of drugs. It has been reported that vitamin E TPGS-coated polystyrene micelles had a 1.4-fold higher cellular uptake than that of the PVA-coated micelles and four to six times higher than that of uncoated micelles using Caco-2 cell line [55]. The studies of mixed TPGS copolymer or TPGS-based micelles for drug delivery are summarized in **Table 3**. The main purposes of TPGS addition are to increase drug loading and to bypass MDR[103]. TPGS itself has limited contribution on the circulation time of micelles. In addition, TPGS shows a great improvement in cell uptake reflected by the enhanced cytotoxicity [13,56], implying its promising potential for oral administration.

3. Factors influencing the properties of polymeric micelles

For peroral delivery, the particle size, surface charge and drug loading of the self-assembled micelles appear to be the most important factors – these can greatly affect the transport route of micelles through the intestinal epithelium membrane and are also crucial to the stability of micelles. Many factors, such as the ratio of hydrophilic and hydrophobic parts on the copolymer, compatibility of the drug with the copolymer and drug loading methods can influence properties of polymeric micelles and therefore their peroral absorption. In this section, these factors are discussed and several drug loading methods are described.

3.1 The ratio of hydrophilic/hydrophobic part

Both drug loading and particle size of polymeric micelles are influenced by the balance of hydrophobic and hydrophilic parts [57]. Copolymers with different hydrophobic modification show three types of behavior in aqueous system upon addition of insoluble drugs as schematically described in **Figure 1**. In general, copolymers with higher hydrophilicity have better compatibility with water molecules and tend to

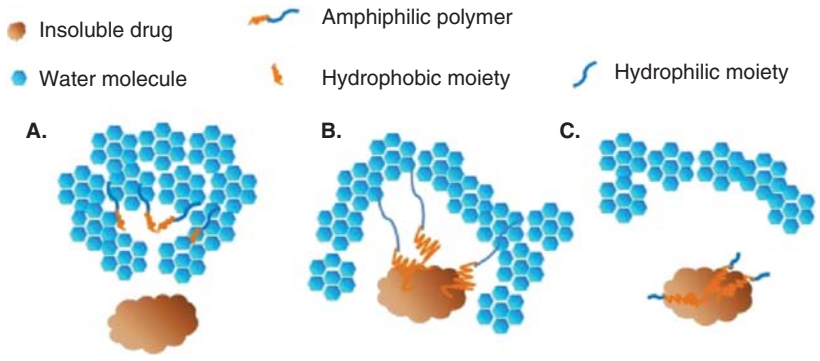

Figure 1. Schematic description of the interaction among amphiphilic copolymers, insoluble drugs, and water molecules: (A) hydrophilicity stronger polymer, **(B)** hydrophilic–hydrophobic balance, **(C)** hydrophobicity stronger polymer.

form self-assembled micelles separated from insoluble drugs (**Figure 1A**). In this case, although it can still encapsulate hydrophobic drugs, the loading capacity is limited and the micelles are less stable. When a huge amount of hydrophobic drugs exist in the system, the polymer may assist the drug to form nanocrystals as a stabilizer [58]. In contrast, polymers with higher hydrophobicity may form aggregates and have a high tendency to precipitate although some of them might absorb on the surface of the drug apart from water molecules (**Figure 1C**). Therefore, to form an optimized micellar system, it is desirable to prepare copolymers with an appropriate hydrophilic–hydrophobic balance to achieve required intermolecular association between drug and water molecules (**Figure 1B**) [59]. For hydrophilic–hydrophobic copolymers, both micelle size and association number decrease with the length of the hydrophobic moiety if the hydrophilic moiety length is fixed and both the size and the association number of the micelles can be varied in predictable ways by changing the temperature, the pH, or the ionic strength of the solution [60]. Similarly, it is reported that the size of the micelle is controlled by both the total copolymer length and hydrophilic-to-hydrophobic block ratio [61]. According to our unpublished data, it was found that the particle size of blank micelles were smaller when the modified polymer had good hydrophilic–hydrophobic balance. It was noted that for a given drug, the extent of incorporation into micelles is a function of factors controlling the micelle size, such as the ratio of hydrophobic to hydrophilic block length. One difference with poor hydrophilic–hydrophobic balanced polymer is that when forming micelles using hydrophilic–hydrophobic balanced copolymer, the particle size shows no significant change upon the increase of drug loading.

In general, the hydrophobic segments of the copolymer are self-associated into a hydrophobic core, while the hydrophilic segments are pushed into water phase surrounding the core [59]. The increase of hydrophobic segments results in stronger hydrophobic interaction and forms tightly packed and thus more stable micellar system [57].

3.2 Concentration of the copolymer

At very low concentration, the copolymers only exist as single chains. As the copolymer concentration increases to CMC, polymer chains start to associate to form spherical micelles and traces of water can be found inside the micellar core, these micelles are described as loose aggregates exhibiting a larger size than the micelles formed at higher concentrations. Because of the larger size and water molecules in the core, these micelles also show poorer stability compared to that of the micelles of a higher concentration. Since the equilibrium will favor micellar formation, micelles will adopt their low energy state configuration and the remaining solvent will gradually be released from the hydrophobic core resulting in a decrease in micellar size [62]. The increase of copolymer concentration results in the decrease of micelles particle size and thus improved stability, because the higher density of hydrophobic segments can lead to a stronger hydrophobic force [63]. If increasing the copolymer concentration further after reaching the saturation to form spherical micelles, the micelles would start changing their shapes to cylinder, bilayer or vesicle causing an increase of particle size and thus a declination of stability [64] and at such a high concentration, a gel-like state may be achieved.

3.3 Drug loading and drug loading method

Encapsulating hydrophobic drugs into micelles may enhance the hydrophobic interaction of drugs with the hydrophobic part of the copolymer, leading to tightly packed and smaller cores of the micelles. For amphiphilic copolymer, as the amount of drug loaded into the hydrophobic cores increased, the particle size of the micelles decreased [63]. After exposing to harsh bioenvironment, loaded drug was slowly released from the micelles, which can be explained by the dissociation of micelles.

Drug loading method is another important factor influencing micelles property. Insoluble drugs can be incorporated into micelles by chemical conjugation, or physical entrapment such as sonication, dialysis and solvent evaporation techniques. Park's group compared the influence of different loading methods on the properties of micelles, indicating that loading

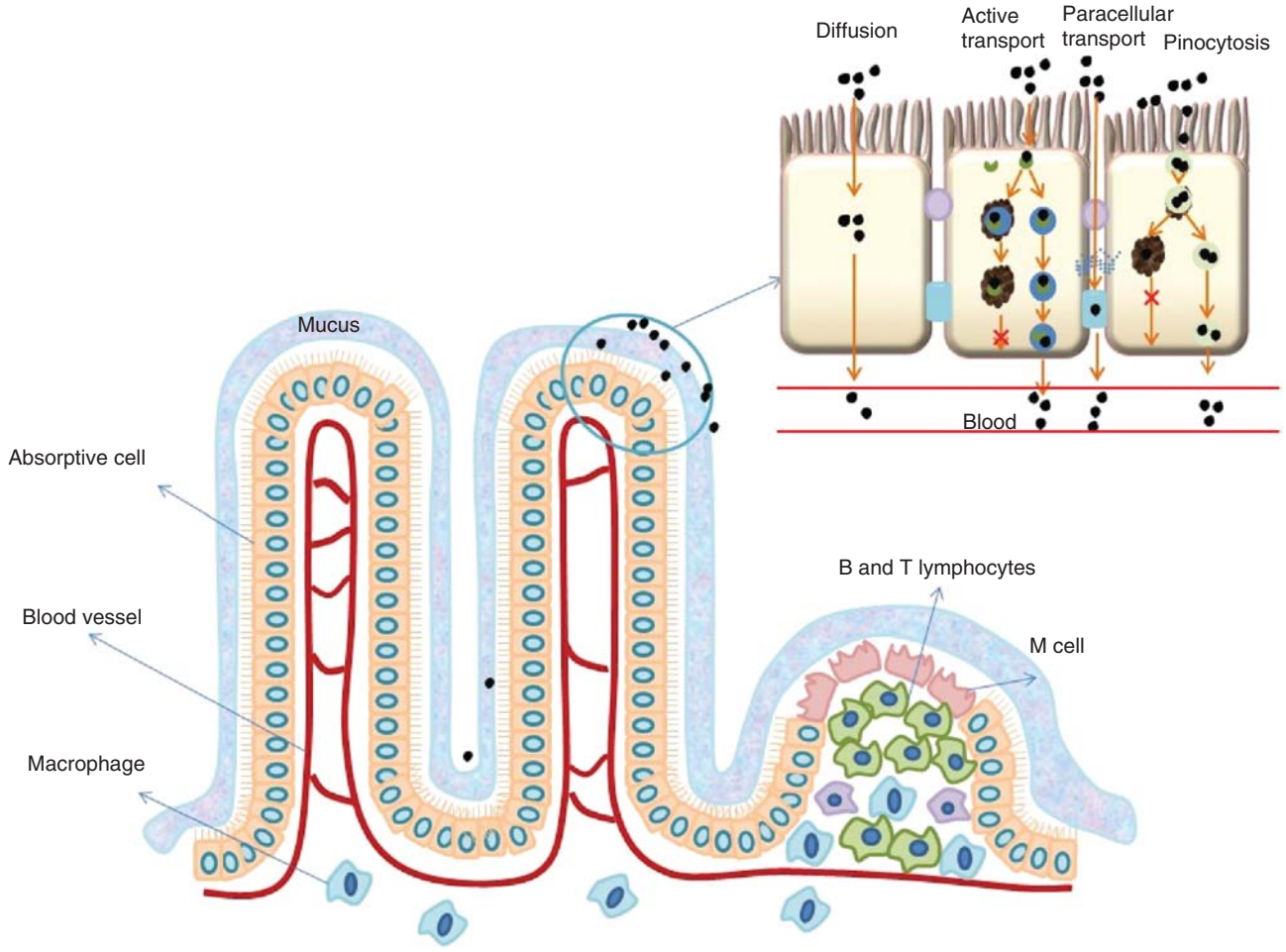

Figure 2. The absorption mechanism of the micelles in the small intestine.

efficiency was significantly dependent on the methods. In their study, the loading efficiency of peptide by solvent evaporation method was higher than that of the sonication method [65]. However, for anticancer drugs, since the compatibility between the drug and the hydrophobic core remarkably influences drug loading capacity, anticancer drug is able to be encapsulated into micelles using sonication method with higher drug loading compared to the other two methods [66]. Furthermore, the sonication method can avoid potential toxic solvent residue and cross-linking agents.

4. Limitations and challenges of micelles

4.1 Low drug loading and encapsulation efficiency

Probably, the most common reason for using polymeric micelles in drug delivery is due to the poor solubility of the drug in aqueous solution. Limited drug loading and low encapsulation efficiency are the major barriers challenging the application of polymeric micelles for oral drug delivery.

One effective strategy to increase drug loading and encapsulation efficiency in the polymeric micelles is to select the copolymers with good compatibility with the drug. Compatibility between a copolymer and the drug is usually calculated by using the following *Hildebrand–Scatchard* equation [67]. Based on the equation, the drug loading in the polymeric micelles can be predicted to some extent [68]. Moreover, improved compatibility can also prevent direct contact of free drug with intestinal mucosa which may lead to toxicity.

In addition to the hydrophobic effect, drug loading into polymer micelles is also facilitated by other interactions between polymers and drugs such as hydrogen bonding and ionic interaction [69,70]. Therefore, another strategy to improve drug loading is to change the nature and the content of the hydrophobic segments [64]. For example, Kim *et al.* prepared nanomicelles by self-assembly in water using hydrophobic cholanic acid-modified chitosan. The anticancer drug cisplatin was able to be encapsulated into the hydrophobic core with a drug loading up to 11.4% [71]. The amphiphilic derivatives of N-octyl-*O*-chitosan prepared by Zhang *et al.* enhanced the solubility of paclitaxel by nearly 1000-fold with a drug loading of 69.9% and encapsulation efficiency of 97.26% [72].

4.2 Poor stability of the micelles

Poor stability of polymeric micelles under physiological condition typically found in the GI tract is another challenge for oral drug delivery. After oral administration, micelles are exposed to pH variation, bile salts and digestive enzymes and can be easily destroyed [73]. It is believed that loading drugs could improve the stability of polymer micelles since lipophilic drugs incorporation lowers the free energy of the system [74]. According to the studies so far, the stability and the integrity of polymeric micelles after oral administration have been proved both *in vitro* and *in vivo*. For example, the *in vivo* imaging in tumor-bearing mice resulted in higher accumulation and deeper penetration into tumor indicating passive targeting drug delivery in animal models [4]. The research proves that polymeric micelles can pass intestinal membrane after oral administration and be absorbed into the blood stream maintaining the integrity of functional micelles. In addition, *in vitro* stability of micelles under different pH conditions was studied [75]. The results showed that chitosan-based micelles are stable maintaining a narrow particle size distribution for 3 days under different pH conditions (7.5, 6.8, and 5.9). Moreover, the results of *in vitro* drug release tests showed that chitosan-based micelles were more stable and even exhibited slower drug release under 1.2 pH conditions compared to that of higher pH conditions [45,76]. Based on the studies so far, the stability of micellar system can be improved by using the following strategies: i) Forming crystallized cores using hydrophobic moieties such as polycaprolactone, contributes to micelle stability and can confer greater drug retention by decreasing the diffusion rate of the drug from the core [77]. ii) Increasing strong cohesive forces between the drug and the polymeric core segments such as hydrophobic force, hydrogen bond, and ionic interaction can confer physical stability to the system [74]. iii) Cross-linking of the shell or core of hydrophobic micelles is another strategy to improve the stability of micelles [78]. iv) Preparing micelles with high surface charge (δ potential > 30 mV) is able to enhance micelle stability as well.

4.3 Barrier to transport through intestinal membrane cells

Another challenge for peroral delivery of polymeric micelles is the limited transport through the biological membrane in the GI tract. The obstacles include the poor stability of the micelles in the hostile acidic environment of the stomach and the high enzymatic activity, the binding of mucous and luminal contents, and the efflux pathways following uptake into epithelial cells [79]. As reported, micelles can be absorbed intactly by either the enterocytes or M cells at the intestinal epithelia via transcellular pathway or paracellular pathway [80], as illustrated in **Figure 2**. Moreover, the concrete absorption route of orally administered micelles was mainly determined by the micellar material and properties of the micelles such as particle size and surface charge [12,55]. The micelles without appropriate membrane transporter can pass the intestinal barrier through paracellular transport or endocytosis. Yuan *et al.* predicted the paracellular permeability of stearic acid-g-chitosan-based micelles across Caco-2 cell monolayer [75]. The result indicated a reversible opening of tight junctions and thus suggested the micelles go across the cell monolayer via the paracellular route. In addition, the uptake of the micelles was partly inhibited at 4°C which confirmed the paracellular transport of chitosan micelles besides endocytosis transport. In addition, many studies have demonstrated that micelles were absorbed by fluid-phase endocytosis (pinocytosis) as a major route [81], an energy-dependent saturable process in which the micelles travelled inside membrane vesicles by partitioning into or out of the lipid bilayers [82,83]. Therefore, based on the transport mechanism of micelles in the GI tract, in addition to increase the stability of polymeric micelles in the specific conditions, several other strategies can be used to enhance the transport of polymeric micelles across the mucosal member, one is to use permeation enhancers [84]. This type of micelles may adhere and infiltrate into the mucus of the intestinal tract through different mechanism, then drug-loaded micelles can permeate into the bloodstream. In any case, safe permeation enhancers should be selected. Fast intestinal transit also limits the drug's bioavailability. The second strategy is to increase the mucoadhesion [85]. The mechanism of mucoadhesion is based on hydrogen bonding such as alginate, polyacrylates and hyaluronic [86], ionic interaction such as chitosan [87] or *van der Waal's* force. It is generally accepted that mucoadhesive polymers can increase drug concentration in the vicinity of the intestinal epithelial cells by intensifying the contact of drug with the intestinal mucosa, and thus can enhance the drug absorption. Multidrug efflux transporters, such as P-glycoprotein (P-gp) and multidrug resistance protein (MRP) located in the apical membrane of enterocytes, limit the oral bioavailability of many anticancer drugs [88]. Therefore, the third strategy is to overcome this absorption hurdle. The micelles formed by some special polymers like pluronic [44] and TPGS [89] have been demonstrated to be effective in delivering drugs across the GI membrane by inhibiting these proteins. In addition, it has been widely reported that inhibition of efflux pumps by multifunctional micelles could lead to enhanced absorption of the drug across the intestine [90]. All of the above-mentioned strategies, including permeation enhancer, increasing the mucoadhesion, inhibiting the function of multidrug efflux transporters, can be achieved either during copolymer preparation process or by adding some excipients in the formulation.

To be emphasized, the transport of polymeric micelles can also be enhanced by using micelles of appropriate particle size. For the general micro/nanoparticles, *in vitro* studies show that the absorption of polymeric particles in the GI tract is particle size-dependent and the absorption of the particles in the diameter range of 50 nm – 500 nm is significantly higher than that of bigger or smaller ones [91]. Based on the fact that micelle is one type of micro/nanoparticles, it can be

assumed that the absorption of polymeric micelles in the GI tract can be remarkably improved by controlling particle size.

5. *In vitro* and *in vivo* behavior of polymeric micelles

Two useful *in vitro* tests are usually used to help predict the *in vivo* behavior of the polymeric micelles: cellular uptake and *in vitro* release [91]. The interaction of the polymeric micelles with cells plays a key role in improving their antitumor behavior *in vivo* and *in vitro*. Recent advances in molecular imaging have provided in depth insight into the micelles' stability and its interaction with cells. The fluorescence resonance energy transfer (FRET) technique, as one of the optical imaging methods, provides the possibility of clarifying the mechanisms of micelles disintegration under physiological conditions and simultaneously elucidating whether the micelles were taken up directly into the cell without dissociation [92,93]. Several studies have evaluated the important factors such as particle size, polymer concentration, temperature, and incubation time on the cellular uptake of the micelles by labeling them using FITC (fluorescein isothiocyanate) [94]. The dependence on both time and concentration demonstrates that the cellular uptake of the micelles is probably caused by adsorptive endocytosis and paracellular transport. Many cellular uptake studies have demonstrated the special advantages of various polymeric micelles such as permeation enhancement, mucoadhesion and inhibition of P-gp mediated efflux, thus may provide sustained delivery of chemotherapeutic agents by oral administration [28,95].

Although *in vivo* behavior of polymeric micelles for oral administration has not been widely investigated so far, several most recent results of the *in vivo* studies provide strong support for the enhanced absorption of polymeric micelles after peroral administration on animal models [4,12,96]. For instance, Yao *et al.* prepared PEG- and TPGS-based polymeric micelles for paclitaxel peroral delivery [4]. The antitumor efficacy results showed a 75% tumor growth inhibition by oral administration higher than that of intravenous injection (25%). The *in vivo* imaging in tumor-bearing mice resulted in higher accumulation and deeper penetration into tumor indicating passive targeting drug delivery in animal models. In addition, Lee *et al.* covalently conjugated docetaxel to chitosan to form self-assembled micelles [96]. The pharmacokinetics of the micelles after oral administration resulted in a sixfold AUC increase compared to that of docetaxel (i.v.). The antitumor efficacy test exhibited a 48% tumor growth inhibition compared to that of 50% in docetaxel (i.v.), and different with injection, no significant body weight change is shown for oral administration. These researches prove that polymeric micelles after oral administration can pass intestinal membrane and be absorbed into the blood stream maintaining the integrity of functional micelles. Moreover, Mo *et al.* demonstrated that polymeric micelles formed by N-octyl-*O*-sulfate chitosan appeared to be an efficient carrier for oral delivery of paclitaxel in rats [12]. It was noted that oral administration of paclitaxel loaded micelles showed a fivefold increase in C_{max}, a sixfold increase in AUC, and a sixfold enhancement in oral availability up to 40.5% compared to that of Taxol [12]. Therefore, it is reasonable to have a positive perspective on the future of polymeric micelles for peroral administration. Anyhow, more *in vivo* tests need to be carried out in this field.

6. Conclusion

During the past decade, a large number of researches have focused on the modification of hydrophilic polymers with functionalized hydrophobic materials, this modification provides a mild and versatile method to form micelles with desirable properties for drug delivery including permeation enhancement, mucoadhesion, inhibition to multidrug efflux transporters. Based on the mechanism of peroral delivery, through modulating or changing the hydrophobic and hydrophilic parts of the copolymer, micelles with various characters could be obtained to overcome the obstacles of peroral delivery. Although holding great promise as a carrier for various poorly soluble anticancer drugs, polymeric micelles for oral administration face several challenges such as low drug loading and encapsulation efficiency, poor stability in the GI tract, and unclarified interaction with GI membrane cells. Despite those barriers, polymeric micelles have nonetheless become promising alternative for peroral delivery of anticancer drugs to reduce toxicity, achieve sustained release, and improve bioavailability.

7. Expert opinion

Amphiphilic polymers are currently under numerous investigations to form self-assembled micelles for successful cancer therapy [74]. However, polymeric micelles as a carrier for peroral administration of anticancer drugs are still a great challenge and several barriers need to be overcome, including low drug loading, poor stability, low transport through the GI membrane, and efflux by some transporters. Moreover, polymeric micelles with sustained release characteristics are desired to further improve patient compliance and decrease related side effect. Nevertheless, based on the results of polymeric micelles for intravenous administration, suitable copolymers with various properties can be chosen to prepare multifunctional polymeric micelles in order to overcome the limitations of peroral chemotherapy. Selecting or synthesizing copolymers with appropriate hydrophile–lipophile balance and having good compatibility with the drug in the meantime are good strategies to increase drug loading and encapsulation efficiency; improved drug loading can also be achieved by changing the nature and content of the hydrophobic segments. The interaction between the copolymer and the drug can be used as a tool to modulate drug release. Stability of the micelles can be improved by forming crystallized cores

using hydrophobic moieties, increasing strong cohesive forces between the drug and the polymeric core segments, cross-linking of the shell or the core of hydrophobic micelles, and preparing micelles with high surface charge. Moreover, during the design of the copolymer structure, prolonged circulation time in the GI tract is one of the most important factors for a successful micellar system for oral delivery since endocytosis and paracellular transport, two main absorption mechanisms of polymeric micelles, require sufficient time and close contact with GI membrane. Fast intestinal transit can also limit drug absorption. Thus, mucoadhesive polymer as basic backbone is essential to form polymeric micelles with long retention time in the GI tract. In addition to mucoadhesion, it is also recommended to take permeability, sustained release, and P-glycoprotein inhibition into consideration. Using safe permeation enhancers is an effective way to increase the transport of polymeric micelles across the mucosal member. It is also important to overcome the absorption hurdle caused by multidrug efflux transporters located in the apical membrane of enterocytes. All of the above-mentioned strategies can be achieved either during copolymer preparation process or by adding some excipients in the formulation. As described in Section 2, structure modification of polyethylene glycol, chitosan, hyaluronic acid, pluronic and tocopheryl polyethylene glycol succinate can form the micelles with the above-mentioned properties and provide the probability of designing multifunctional micelles in order to enhance absorption of the drug across the intestine mucosa. Furthermore, both the copolymer structure and the drug loading method should be controlled in order to get polymeric micelles with particle size less than 500 nm and improved stability for better absorption.

At present, although some *in vitro* studies have been carried out for the preparation and the characterization of polymeric micelles for peroral delivery of poorly soluble anticancer drugs, only limited information is available regarding *in vivo* behavior, which showed successful transport through GI membrane into blood stream further reaching tumor cells [4,12]. Extensive studies in this field especially pharmacokinetics and antitumor efficacy are required. *In vitro* and *in vivo* correlation should be established to ease the quality control and the formulation optimization. Still, the bioavailability of some polymeric micelles is still quite low after peroral administration, some novel strategies need to be taken to further increase their absorption across the intestinal membrane with high safety for the promise of clinical application. In addition, it is desirable to provide intestinal toxicity results after the antitumor efficacy test of drug-loaded polymeric micelles in animal models after peroral administration.

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Declaration of interest

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