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Zwitterionic polymers: Addressing the barriers for drug delivery

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ABSTRACT

Nanocarriers play an important role in drug delivery for disease treatment. However, nanocarriers face a series of physiological barriers after administration such as blood clearance, nonspecific tissue/cell localization, poor cellular uptake, and endosome trapping. These physiological barriers seriously reduce the accumulation of drugs in target action site, which results in poor therapeutic efficiency. Although polyethylene glycol (PEG) can increase the blood circulation time of nanocarriers, its application is limited due to the "PEG dilemma". Zwitterionic polymers have been emerging as an appealing alternative to PEG owing to their excellent performance in resisting nonspecific protein adsorption. Importantly, the diverse structures bring functional versatility to zwitterionic polymers beyond nonfouling. This review focuses on the structures and characters of zwitterionic polymers, and will discuss and summarize the application of zwitterionic polymers for drug delivery. We will highlight the strategies of zwitterionic polymers to address the physiological barriers during drug delivery. Finally, we will give some suggestions that can be utilized for the development of zwitterionic polymers for drug delivery. This review will also provide an outlook for this field. Our aim is to provide a comprehensive and systemic review on the application of zwitterionic polymers for drug delivery and promote the development of zwitterionic polymers.

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1. Introduction

Drug therapy is one of the most promising strategies for disease treatment. Various kinds of drugs have been discovered and applied such as small molecule drugs, nucleic acid drugs, protein and peptide drugs [1–3]. However, the therapeutic efficiency of free drugs is always limited due to their intrinsic disadvantages such as instability, short half-life in blood or poor cellular uptake [4–7]. Nanocarriers are promising strategy to deliver the drug to the target action site [8–13]. Various kinds of nanocarriers, such as liposomes, micelles, vesicles, nanogels, exosomes, mesoporous silica nanoparticles (MSNs) and metal-organic frameworks (MOFs), have been developed [14–17]. These nanocarriers have several advantages such as tunable size, diverse shape and facile functionalization [18–21]. Despite the great potential, nanocarriers still face multiple substantial challenges. To gain access to the target action site, nanocarriers have to hurdle a series of physiological barriers after administration such as blood clearance, nonspecific tissue/cell localization, poor cellular uptake, and endosome trapping [22–25]. Development of materials for overcoming these hurdles is urgently needed to enable future success in drug delivery and therapy.

Polyethylene glycol (PEG) is an important type of hydrophilic polymer that is always utilized to overcome the physiological barriers [26]. The attachment of PEG to the surface of nanocarriers, known as "PEGylation", has been a "gold standard" strategy to resist nonspecific protein adsorption [27]. PEG can form hydration layer on the surface of nanocarriers *via* hydrogen bond. The hydration layer formed by PEG is believed to improve the hydrophilic property and stability of nanocarriers [28]. PEGylation can resist nonspecific protein adsorption and increase the circulation time of nanocarriers in blood [29]. However, the application of PEG is limited due to the "PEG dilemma". PEGylated nanocarriers lose their long circulation property after repeated intravenous injection because of the accelerated blood clearance (ABC) phenomenon due to the generation of anti-PEG immunoglobulin antibodies [30–34]. In addition, PEG shells can hinder the interaction between nanocarriers and cell membranes, which severely reduce the endocytosis and endosomal escape efficiency of drugs [35]. Furthermore, PEG is reported to be easily destroyed by oxidation in the presence of transition metal ions and oxygen, which severely impairs the antifouling property of PEG during prolonged usage or storage [36]. Therefore, it is urgently needed to develop alternative materials to

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address the physiological barriers during drug delivery and further enhance the therapeutic efficiency.

In recent decades, zwitterionic polymers, bearing an equal number of positive charges and negative charges, have been emerging as an appealing alternative to PEG owing to their excellent performance in resisting nonspecific protein adsorption [37–40]. Zwitterionic polymers such as poly(carboxybetaine) (PCB), poly(sulfobetaine) (PSB) and poly(phosphorycholine) (PPC) have been widely utilized in nanocarriers [41]. It has been found that nanocarriers with zwitterionic polymers show prolonged blood circulation time, reduced immune response, and negligible ABC phenomenon after administration [42–44]. Moreover, zwitterionic polymers have more properties to overcome the multiple physiological barriers *in vivo* due to their unique and diverse structures. Nowadays, zwitterionic polymers have been widely used in drug delivery due to their specific properties and functions. This review focuses on the structures and characters of zwitterionic polymers, and will discuss and summarize the application of zwitterionic polymers for drug delivery. We will highlight the strategies and applications of zwitterionic polymers to overcome the physiological barriers during drug delivery. Finally, we will give some suggestions that can be utilized for the development of zwitterionic polymers for drug delivery. This review will also provide an outlook for this field. Our aim is to provide a comprehensive and systemic review on the application of zwitterionic polymers for drug delivery and promote the development of zwitterionic polymers.

2. Structures and characters of zwitterionic polymers

Zwitterionic polymers are entirely hydrophilic, and are considered as an alternative to the widely used PEG polymers for nonfouling due to their strong ability of resisting nonspecific protein adsorption [37,45]. Different from PEG polymers that sharing the same repeating unit, zwitterionic polymers, as biomimetic polymers, have distinctive monomeric chemical structures that are inspired from natural biological components. Zwitterionic polymers, containing an equimolar number of cationic and anionic terminal groups, maintain the overall charge as neutral [46,47]. As shown in Fig. 1, the cationic groups of zwitterionic polymers mainly include protonated amino and quaternary ammonium, and the anionic groups mainly include carboxylate, sulfonate and phosphate group [48,49]. Therefore, in comparison with PEG with no charge group, zwitterionic polymers possess high dipole moments and comprise highly charged groups. Based on the differences of zwitterionic side chains, the zwitterionic polymers can be mainly divided into two categories [50,51]. The first category is the monomers that carry both a cationic group and an anionic group together in one unit. These monomers include carboxybetaine (CB), sulfobetaine (SB), phosphorylcholine (PC) and amino acid. The second category, also called "mixed charge", is the zwitterionic polymers that carry 1:1 positive and negative charges on different monomer units. These zwitterionic polymers can be obtained by a homogeneous mixture of 1:1 oppositely charged monomers in a copolymerization reaction. As shown in Table 1, the diverse structures of zwitterionic polymers make them have diverse and unique characters that are suitable for drug delivery.

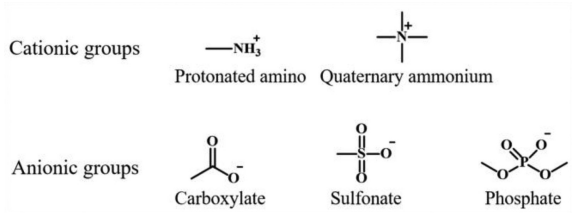

Fig. 1. Representative structures of cationic groups and anionic groups in zwitterionic polymers.

Table 1
The characters of zwitterionic polymers.

Zwitterionic polymer	Character	Refs.
PCB	Monomer structure similar to glycine betaine Carboxylate groups for modification pH-sensitive and protonation	[54–61]
PSB	Responding to ionic strength Thermo-sensitive and exhibiting an UCST	[64–67]
PPC	Monomer structure similar to the headgroup of PC lipids in the outer surface of living cells Conformation of PMPC affected by salts through ionic interaction	[74–79]
PMCP	Reverse structure of PC Strongly binding to a variety of cell membranes	[80–85]
Poly(amino acids)	Biodegradability and bioactive functionality Various side groups suitable for modification Hydration ability affected by kinds of amino acids	[86–92]

2.1. Poly(carboxybetaine)

In the family of zwitterionic polymers, PCB, PSB and PPC are the most extensively explored and applied due to their good nonfouling property, which is attributed to their strong hydration capacity [52]. The monomer of zwitterionic PCB has a cationic quaternary ammonium group and an anionic carboxylate group. The monomer structure of PCB is similar to that of glycine betaine, which is vital to osmotic regulation in living organisms. The amounts of glycine betaine intake by humans are about 0.1 g to 2.5 g per day [53]. Therefore, biomimetic PCB has good biocompatibility. In addition, PCB possesses a plentiful of carboxylate groups, which makes it easy to bind functional molecules such as targeting ligands or active biomolecules *via* conjugation chemistry without disrupting the resistance after modification [54–56]. Cao and co-workers conjugated the amine groups of the galactose ligands with the carboxylate groups of PCB on the surface of PLGA-PCB/NBD nanoparticles (NPs) through 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide and *N*-hydroxysuccinimide (EDC/NHS) chemistry [57]. Galactose, a ligand targeting the asialoglycoprotein receptors in hepatoma cell lines, could enhance the cell-binding ability of NPs. In our previous work, we synthesized a polyprodrug amphiphiles PCB-Se-Se-simvastatin by conjugating hydrophobic drug simvastatin to part of the carboxylate groups of PCB *via* reactive oxygen species (ROS)-labile diselenide bond [58]. The hydrophobic polyprodrug unit of PCB-Se-Se-simvastatin was positively charged, and could simultaneously load hydrophobic superparamagnetic iron oxide nanocubes (SPIONs) and absorb negatively charged let-7b antisense oligonucleotide. The loading content of simvastatin was 34.41% for the CSeM/let-7b NPs with PCB-Se-Se-simvastatin due to their minor inert materials, which was significantly higher than that of CH/M/let-7b NPs encapsulating simvastatin.

Moreover, the carboxylate groups (-COO^-) of PCB are pH-sensitive, and can be protonated and become carboxyl group (-COOH) at acidic environment [59]. After protonation, the negative charges of PCB are removed and PCB changes from neutral to positively charged, which can be utilized to load negatively charged drugs such as nucleic acid drugs and protein/peptide drugs [60]. In our previous work, small interfering RNA (siRNA) siSOX9 was

loaded onto the NPs with PCB at acidic environment [6]. As expected, the complexing ability was enhanced under lower pH conditions, whereby NPs achieved complete retardation at an N/P ratio of 3:1 at pH 4, whereas it was at an N/P ratio of 15:1 at pH 6. The NPs with siSOX9 combined with neural stem cells (NSCs) formed an effective therapeutic system to alleviate Alzheimer's disease (AD). In addition, we developed oral insulin PCB/INS particles through the electrostatic interaction between positively charged PCB at pH 5.0 and negatively charged insulin in 0.01 mol/L NaOH [61]. The loading efficiency reached above 86% at the mass ratio of 8:1 between PCB and insulin. PCB/INS particles achieved sustained release of insulin at pH 7.4 due to their charge-switchable ability. Considering the large pH gradient between the stomach and the intestine, PCB would provide a platform that controls the retention and release of drugs to small intestine, which is the site of most efficient adsorption in the gastrointestinal (GI) tract after oral administration [62]. In addition, the anionic carboxylate groups of PCB/INS particles could induce the open of the tight junctions of intestinal epithelium in endocytosis-mediated lysosomal degradation pathway, which resulted in increased intestinal permeability of insulin for highly efficient type 1 diabetes mellitus (T1DM) treatment [61]. Thus, PCB could provide a promising platform for oral protein and peptide drug delivery.

2.2. Poly(sulfobetaine)

Zwitterionic PSB has a cationic quaternary ammonium group and an anionic sulfonate group [63]. PSB is attracted more attention because of not only its excellent nonspecific protein adsorption and biocompatibility, but also responding to external stimuli such as temperature and ionic strength [64]. PSB can expand in saline solution compared to pure water, which would trigger the drug release. In addition, PSB is a thermo-sensitive polymer and exhibits an upper critical solution temperature (UCST) in aqueous solution [65]. When the temperature is higher than the UCST, PSB will expand and swell up from a state of collapsed globule [66]. The UCST of PSB is usually in the range of 35–40 °C, and can be controlled *via* its molecular weight, copolymerization, *etc*. [67]. Therefore, PSB can be utilized to prepare salt- and thermo-responsiveness drug delivery systems. Sun and co-workers constructed a novel zwitterionic nanocapsules (ZNCs) with PSB by inverse reversible addition fragmentation transfer (RAFT) miniemulsion interfacial polymerization [68]. The PSB chains in the shell of the ZNCs were bonded together tightly in water. The ZNCs exhibited a salt-induced swelling behavior that the ZNCs were in a swelling state once in saline solution. The hydrodynamic diameters of ZNCs-10 wt% (1:1) increased from 200 nm in water to 478.27 nm in 4.0 mol/L NaBr solutions. Furthermore, the ZNCs also showed thermo-responsiveness due to the phase transition of PSB above its UCST. The hydrodynamic diameters of the ZNCs-0 wt% (1:1) increased from 25 °C to 70 °C, especially around 45 °C to 50 °C. The swelling of nanocarriers would achieve controlled release of drugs. Therefore, the PSB provides a platform for constructing salt- and thermo-responsive nanocarriers.

2.3. Poly(phosphorycholine)

Zwitterionic PPC shows excellent hydrophilicity, biocompatibility, stability and bioavailability [69–72]. PPC has a cationic quaternary ammonium group and an anionic phosphate group [73]. Owing to its similar structure to the headgroup of PC lipids in the outer surface of living cells, the PPC polymers could serve as the biomimetic nanocarriers [74–77]. Poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC) has been approved by the Food and Drug Administration (FDA) in 2015 [59,78]. Cai *et al.* synthesized poly(ε-caprolactone)-*b*-PMPC (PCL-PMPC) that self-assembled into small and uniformed micelles for the delivery of doxorubicin (DOX) [79]. The PCL_{20}-$PMPC_5$ and PCL_{20}-$PMPC_{10}$ micelles had longer elimination half-life of 30.21 ± 9.75 h and 41.62 ± 9.16 h, respectively, while it was 10.11 ± 1.16 h for PCL-PEG micelles. In addition, PMPC could accelerate the DOX release at pH 5.5 as the conformation of PMPC was affected by salts in the solution through ionic interaction. With these advantages and better tumor accumulation, the PCL-PMPC micelles showed better therapeutic efficacy than DOX-loaded PCL-PEG micelles.

On the other hand, poly(2-methacryloyloxyethyl choline phosphate) (PMCP) has been developed as a novel antifouling zwitterionic polymer, which bears choline phosphate (CP) [80,81]. The CP is the reverse structure of PC [82]. Traditional antifouling materials inhibit protein adsorption and cell adhesion. Different from traditional antifouling materials, PMCP achieves antifouling and promoted cell adhesion simultaneously [83]. Yun and co-workers demonstrated that hyperbranched polyglycerols (HPGs) decorated with polyvalent CP groups could strongly bind to a variety of cell membranes mediated by the interaction between the multivalent CP and PC groups [84]. Moreover, PC-rich membranes of Chinese hamster ovary (CHO) cells adsorbed and rapidly internalized fluorescent HPG-CP but not HPG-PC molecules. Wang and co-workers synthesized PMCP-DOX conjugate system, and found that the PMCP-DOX could be rapidly and efficiently internalized by various tumor cells such as MCF-7, A549 and HepG2 due to the strong interaction between multivalent CP groups and cell membranes [85]. These researches suggested that CP-decorated polymers or nanocarriers could be used as drug delivery agents and tissue engineering.

2.4. Poly(amino acids)

Amino acids are natural zwitterions, composed of an asymmetric α carbon at their center, an amine group, a carboxyl group, a hydrogen atom, and a side chain specific to each amino acid [86]. The unique zwitterionic and biomimetic nature of amino acids has promoted researchers to design new zwitterionic antifouling polymers incorporating amino acids [87]. Zwitterionic poly(amino acids) and their derivatives have good biocompatibility and biodegradability, and show good antifouling property similar to that of zwitterionic PSB and PCB due to their strong hydration ability [88]. In addition, the various side groups of zwitterionic poly(amino acids) and their derivatives are suitable for further functionalization. Therefore, zwitterionic poly(amino acids) and their corresponding derivatives have been extensively studied in drug delivery systems due to these advantages [89]. Several amino acids derived zwitterionic polymers including poly(serine methacrylated) (pSerMA), poly(lysine methacrylamide) (pLysAA), poly(ornithine methacrylamide) (pOrnAA), poly(glutamate) (pGlu) poly(*N*4-(2-methacrylamidoethyl) asparagine) (pAspAA) and poly(*N*5-(2-methacrylamidoethyl) glutamine) (pGluAA) have been developed and applied [90,91]. The kinds of amino acids directly impact the ability of creating a hydration layer around the zwitterionic poly(amino acids), which is essential for the stealth property. Banskota and co-workers designed zwitterionic poly(amino acids) with a repetitive $(VPX_1X_2G)n$ motif, where X_1 and X_2 were cationic and anionic amino acids, respectively [92]. They showed that $(VPKEG)_{120}$, a combination of lysine (K) and glutamic acid (E) in the zwitterionic poly(amino acids), conferred better pharmacokinetics with half-life of 12.0 ± 0.4 h after intravenous administration and 15.6 ± 0.6 h after subcutaneous administration. Besides hydration ability, different kinds of amino acids might give different bioactive functionality to zwitterionic poly(amino acids). Therefore, zwitterionic poly(amino acids) would be a promising platform for drug delivery due to their biocompatibility and diversity.

3. Addressing the physiological barriers for drug delivery

Physiological barriers prevent successful accumulation of drugs specifically at diseased and action sites. Substantial research efforts have been devoted to incorporating multiple moieties within a single nanocarrier to give multiple functionalities. However, many of these strategies fail to adequately address these physiological barriers, and the complexity results in failure of clinical translation. Due to their special structures, zwitterionic polymers are promising candidates for the hydrophilic components of drug delivery system. Benefitting from their unique structures with both cationic and anionic groups alone, zwitterionic polymers are capable of forming hydration layer on the surface of nanocarriers *via* strong electrostatic interaction rather than the hydrogen bonding of PEG [49,68]. Nanocarriers with zwitterionic polymers exhibit outstanding resistance to nonspecific protein adsorption [93,94]. Therefore, nanocarriers coated with zwitterionic polymers show prolonged blood circulation time and negligible immune response. Especially, as shown in Fig. 2, the diverse structures bring functional versatility to zwitterionic polymers beyond nonfouling. Zwitterionic polymers can overcome physiological barriers including blood clearance, nonspecific tissue/cell localization, poor cellular uptake, and endosome trapping during drug delivery. Nanocarriers with zwitterionic polymers exhibit great biostability, biocompatibility, and enhanced therapeutic efficacy.

3.1. Prolonged blood circulation time

The pharmacokinetics of nanocarriers *in vivo* is one of the key parameters for successful drug delivery. The body has a sophisticated immune system that can recognize the non-self materials as invaders and eliminate them by the mononuclear phagocyte system (MPS) [95]. The MPS is composed of a system of phagocytic cells, mainly resident macrophages in the spleen, lymph nodes and liver. The MPS can sequester nanocarriers immediately after injection to clear the nanocarriers from blood circulation [96]. The clearance process begins with opsonization of nanocarriers, involving the adsorption of plasma proteins such as serum albumin, apoliproteins, complement components and immunoglobulins onto

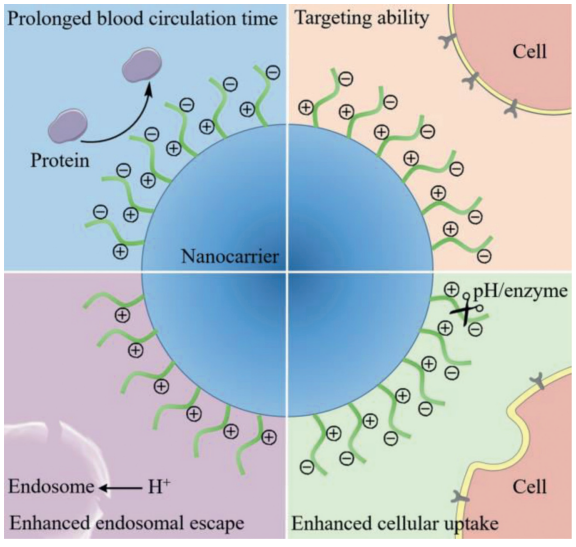

Fig. 2. Zwitterionic polymers addressing the physiological barriers for drug delivery. Zwitterionic polymers can prolong the blood circulation time of nanocarriers by resisting nonspecific protein adsorption. Zwitterionic polymers have specific cell targeting ability due to their biomimetic structure. The pH/enzyme-triggered charge reversal of zwitterionic polymers enhance the cellular uptake and the protonation of zwitterionic polymers enhance the endosomal escape of drugs.

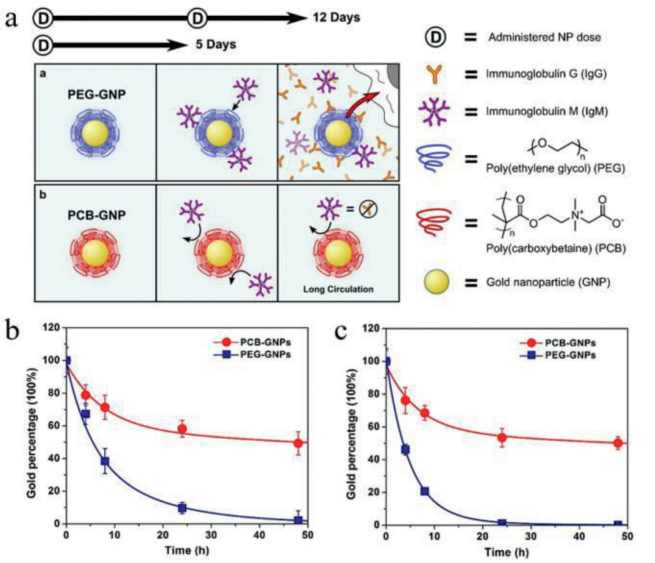

Fig. 3. (a) Schematic illustrations of the sequence of events after PEG-coated GNPs and PCB-coated GNPs enter the blood stream. *In vivo* circulation time of the GNPs after the (b) first and (c) second administration. Reproduced with permission [104]. Copyright 2014, Elsevier.

the surface of circulating nanocarriers [24]. Most of the unprotected nanocarriers can be cleared from the blood circulation by the MPS within seconds to minutes after intravenous administration [97]. Therefore, it is important to develop materials that endow nanocarriers with stealthy property to resist the nonspecific plasma proteins adsorption and aid the nanocarriers escape the clearance by MPS, thereby reaching the desired target sites ultimately.

Nonfouling surface is a key feature for long blood circulation of NPs. By 2008, zwitterionic polymers had been shown to resist nonspecific protein adsorption [98]. Zwitterionic polymers have been found to provide stronger hydration *via* electrostatic interaction, and are ultralow fouling against adsorption and attachment from proteins [99–101]. Wu *et al.* found that about eight water molecules were tightly bound with one SB unit, while only one water molecule for ethylene glycol (EG) [102]. The hydrated water molecules on the SB unit were more tightly bound than on the EG unit before saturation. PCB demonstrated extremely low protein adsorption ($<0.3\,ng/cm^2$) against undiluted human serum or plasma [103]. The extremely low fouling almost does not influence the blood circulation time (Fig. 3a). It has been shown that PCB-coated gold NPs (GNPs) had superior blood retention compared to PEG-coated GNPs. The elimination half-life of PCB-coated GNPs was 243.9 h after the first intravenous injection, while it was only 10.7 h for the PEG-coated GNPs (Fig. 3b). Therefore, the ultrahigh nonfouling properties make zwitterionic polymers a promising alternative to conventional PEG to prolong the circulation time of nanocarriers in blood [105–107].

Importantly, it has been demonstrated that zwitterionic polymers can avoid ABC phenomenon [108]. In our previous works, we found that zwitterionic PCB-coated liposomes with small molecule drug topotecan or siRNA did not induce the ABC phenomenon [109,110]. As shown in Fig. 3, Yang *et al.* also demonstrated that PCB-coated GNPs did not induce the ABC phenomenon [104]. After the second dose, the elimination half-life was 268.0 h and 8.2 h for PCB-coated GNPs and PEG-coated GNPs, respectively (Fig. 3c). PCB-coated GNPs showed little change between the first and second doses, while PEG-coated GNPs suffered ABC phenomenon. She and co-workers reported a hypoxia-degradable zwitterionic PPC nanogels named as HPMPC for glioblastoma treatment [111]. After

intravenous injection, the HPMPC nanogels presented super blood circulation than POEGMA due to the stronger antifouling ability. Moreover, the blood circulation curves and IgM level of HPMPC nanogels remained almost unchanged between the first and second administration, which indicated that the repeated injection of zwitterionic PPC nanogels did not cause ABC phenomenon. Men and co-workers developed zwitterionic nanogels based on poly(sulfobetaine methacrylate) (PSBMA) for reduction-responsive DOX delivery to tumors. PSBMA nanogels displayed long circulation in blood after intravenous injection, and a small change was found in half-life of nanogels between the first (34.1 h) and the second injection (30.5 h), indicating that there was no ABC phenomenon for the PSBMA nanogels [112].

In addition, the hydration capacity along with the nonfouling property of zwitterionic polymers increases as the intramolecular distance between the positively and negatively charged site of the zwitterionic headgroups decreases. On the basis of this rule, Li *et al.* synthesized trimethylamine *N*-oxide (TMAO)-derived zwitterionic polymer (PTMAO) as a new class of ultralow fouling biomaterials [113]. TMAO is a small organic osmolyte that presents in saltwater fishes. TMAO might be an excellent zwitterionic headgroup for nonfouling material as its positively charged groups are directly connected with the negatively charged groups. Compared with the native uricase ($T_{1/2} = 3.9$ h), PEG-uricase extended the circulation time to 16.2 h after a single injection, while its $T_{1/2}$ shrunk to 10.1 h after five administration. In contrast, PTMAO-uricase exhibited a persistently superb circulation half-life as long as 19.1 h after the first dose and 18.2 h after the third dose. Therefore, PTMAO not only could extend the blood circulation time of uricase, but also could avoid ABC phenomenon. In addition, the PTMAO had a stabilizing effect on the uricase under high stress and temperature condition, which might be ascribed to the protein-stabilizing effect of TMAO. Therefore, the discovery of PTMAO polymers would provide a powerful new biomimetic material with excellent nonfouling and protein stabilizing ability.

Most studies focused on the ultrahigh nonfouling properties of zwitterionic polymers for prolonged blood circulation time of nanocarriers. Interestingly, some studies have found that some zwitterionic polymers could extend the blood circulation time *via* bioactive functionality. Chen and co-workers developed a phospholipid-binding zwitterion, poly(2-(*N*-oxide-*N*,*N*-diethylamino)ethyl methacrylate) (OPDEA), that is not sticky towards proteins and can also bind the cell membrane of red blood cells due to the interaction between *N*-oxide and the hydrophilic heads of phosphatidylcholine and phosphatidylethanolamine [114]. The conjugates of small molecule anticancer drug 7-ethyl-10-hydroxycamptothecin (SN38) with the OPDEA had long blood circulation half-life with similar to those of PEG-SN38, and OPDEA-SN38 had faster in distribution and slower in later clearance than PEG-SN38. As the binding was reversible with tumor endothelial cells and cancer cells, the OPDEA-RhoBPSN38 was able to reach the hypoxia region through transcytosis-mediated extravasation into tumor interstitium and infiltration into tumor, leading to the eradication of large tumors against subcutaneous HepG2 xenograft tumors, orthotopic HepG2-Luci tumors and the liver cancer patient-derived tumor xenografts in mice. Therefore, the OPDEA would provide a simple and promising platform for anticancer drug delivery.

3.2. Targeting ability

With long blood circulation, nanocarriers are expected to undergo cellular internalization to release the drugs to exert therapeutic effects on cytoplasmic and nucleus targets. To enhance the drug accumulation in target tissues or cells, nanocarriers should have target ability to recognize the specific type of cells. Receptor-mediated delivery has been considered as a powerful strategy to promote the transport efficacy of nanocarriers [115]. Various receptors are found expressed on the specific cells such as transferrin receptor, low-density lipoprotein receptor and nicotinic acetylcholine receptor [116,117]. However, the ligands are always needed to be modified on the surface of the hydrophilic segment of nanocarriers to target the receptors. The multistep modification processes make the preparation process complicated and prevent the clinical translation. One promising strategy to overcome this issue is to find the hydrophilic materials with nonfouling and targeting ability.

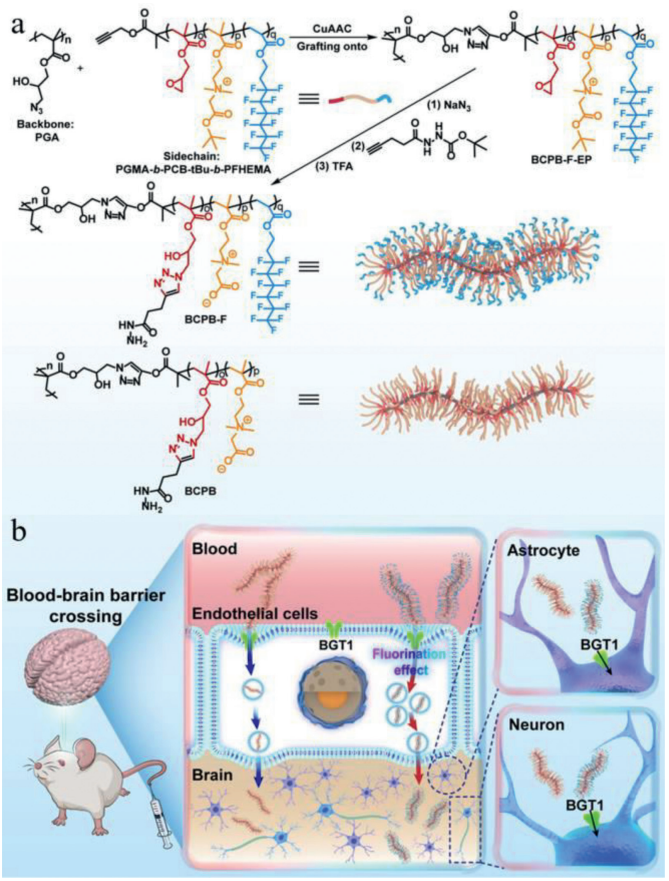

Fig. 4. (a) Synthesis route of BCPB-F and BCPB. (b) Schematic diagram of the BBB penetration of BCPB-F and BCPB. Reproduced with permission [123]. Copyright 2022, WILEY-VCH Verlag GmbH & Co.

As hydrophilic polymers, some zwitterionic polymers have specific cell targeting ability due to their biomimetic structure. Betaine is a methyl derivative of glycine and plays an important role in cellular osmoregulation and metabolism of human physiology [118]. More importantly, betaine is a specific substrate of BGT1 that is expressed at blood-brain barrier (BBB) and mediates the transport of betaine. For brain diseases, BBB seriously impedes the effective therapy by restricting the permeability of nanocarriers [119–122]. The monomer of PCB has the structure similar to the betaine. Therefore, PCB can pass the BBB *via* a betaine transporter BGT1. As shown in Fig. 4, Wang and co-workers synthesized a DOX-loaded fluorinated cylindrical polymer brushes (CPBs) named BCPB-F-DOX with a triblock sidechain containing a PCB block and a fluorinated block [123]. The DOX concentration in brains at 72 h postinjection of BCPB-F-DOX and BCPB-DOX without fluorinated segments were 0.50% ± 0.15% and 0.26% ± 0.02% ID/g, respectively, while the DOX concentration in brains after injecting ECPB-DOX with PEG segments instead of PCB segments was too low to be determined ac-

curately. The results demonstrated that PCB had a potent capacity for transporting drugs to brain.

In addition, proton-assisted amino acid transporter 1 (PAT1) can facilitate the penetration of betaine and betaine derivatives through the intestinal epithelial cell layer. The intestinal epithelial cell layer is one of the key players of intestinal adsorption [124,125]. The intestinal epithelial cell layer can prevent foreign particles from entering into blood [126]. Oral administered drugs can be absorbed into the blood only after nanocarriers crossing the intestinal epithelial cell layer [127–129]. Han and co-workers reported an insulin-loaded micelle platform composed of 1,2-distearoyl-*sn*–glycero-3-phosphoethanolamine (DSPE)-PCB (DSPE-PCB) [130]. A significantly higher-level cellular uptake of the DSPE-PCB micelle particle was observed on Caco-2 cells (human colon epithelial cells known for PAT1 overexpression) compared with regular 3T3 cells. The cellular uptake of DSPE-PCB micelle particle by Caco-2 cells was significantly inhibited with the presence of PAT1 substrates betaine or L-tryptophan. The zwitterionic micelle enabled insulin penetration through the mucus and efficient PAT1-mediated epithelial absorption. The oral bioavailability of the DSPE-PCB/insulin enteric-capsule was as high as 42.6%. Zhou and co-workers engineered a pH-triggered self-unpacking capsule encapsulating zwitterionic hydrogel-coated MOF NPs for oral delivery of exendin-4 (Ex@MIL101@Gel±@Cap) [131]. The zwitterionic hydrogel layer based on [3-(methacryloylamino)propyl]dimethyl(3-sulfopropyl)ammonium hydroxide inner salt (MPDMSA) imparted unique capability of permeation across the mucus layer due to its nonfouling performance and effective internalization by epithelial cells mediated by the PAT1. Notably, after the oral administration of Ex@MIL101@Gel±@Cap in rats with diabetes, a remarkable hypoglycemic effect was obtained with a relative pharmacological availability 17.26% ± 9.8%. Therefore, the zwitterionic polymers PCB and PSB was able to address the epithelial barriers, and had the potential to be a practical solution for oral drug delivery.

Different from zwitterionic polymers PCB and PSB, the monomer of PPC contains a choline and acetylcholine analogue. Choline transporters and acetylcholine transporters are extensively expressed in the luminal brain capillary endothelial cells and nervous system [132]. Choline is actively transported from the circulating blood to the brain through choline transporters for the synthesis of acetylcholine and other molecules. As-synthesized acetylcholine is stored and transported within the vesicles of neuron cells through acetylcholine transporters. Therefore, with the structure similar to the choline and acetylcholine, PPC may interact with choline transporters and nicotinic acetylcholine receptors in a similar way to choline and acetylcholine for the treatment of central nervous system diseases. In our previous study, we developed self-catalytic siRNA nanocarrier (S/Ce-PABMS) with the modification of PMPC (Fig. 5) [133]. Compared with S/Ce-PABES modified with PEG, S/Ce-PABMS significantly increased the Cy5 fluorescence of Cy5-siRNA in bEnd.3 cells and SH-SY5Y cells (Fig. 5c). Moreover, the addition of PMPC inhibited the cellular uptake of S/Ce-PABMS in both cells, indicating that PMPC could mediate the S/Ce-PABMS to penetrate the BBB and accumulate in the neuron cells. *In vivo* biodistribution of S/Ce-PABMS showed the same trend after intravenous injection, and the NPs significantly improved dyskinesia of Parkinson's disease (PD) mice. Therefore, the PMPC would provide a promising platform for central nervous system disease therapy. Zwitterionic polymers do not have targeting ability for all organs or tissues. Under this condition, the surface decoration of targeting ligands is always used for these nanocarriers [57,134,135].

3.3. Enhanced cellular uptake

Cellular uptake is critical to the success of drug delivery, considering that nanocarriers have to deliver drugs to the cytosol, nucleus, or other specific intracellular sites [136]. Many types of developed nanocarriers cannot be successfully applied owing to the poor cellular uptake. For most nanocarriers, the efficient cellular uptake is vital for achieving high-yield therapeutic efficacy. However, PEGylation reduces the interaction between nanocarriers and cell membranes required for cellular uptake due to the steric hindrance [35,137]. In comparison, nanocarriers with zwitterionic polymers have better cellular uptake than that with PEG. As shown in Fig. 6, Jackson *et al.* found that siRNA polyplexes with 20 kDa PMPC had comparable blood circulation half-life with 20 kDa PEGylated siRNA polyplexes, while zwitterionic 20 kDa PMPC showed significantly higher tumor cell uptake level than 20 kDa PEGylated polyplexes after 24 h of accumulation time in tumor-bearing mice [138]. The excellent pharmacokinetics and cellular uptake of 20 kDa PMPC siRNA polyplexes resulted in the highest *in vivo* luciferase silencing (>75% knockdown for 10 days with single intravenous in-

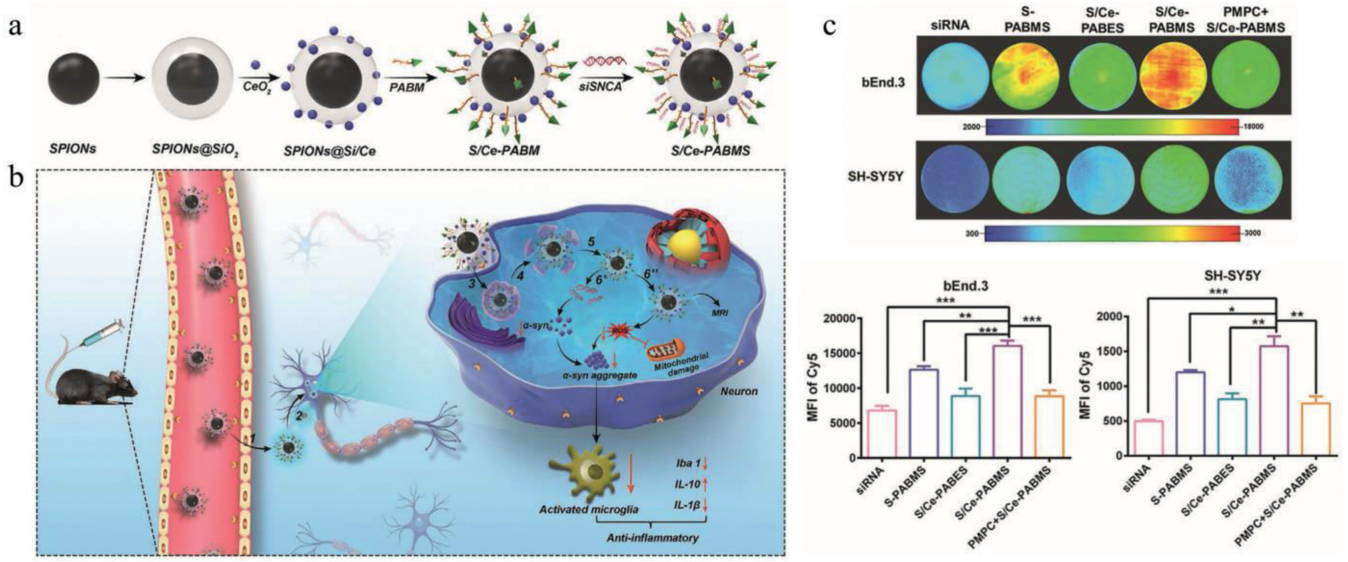

Fig. 5. (a) Preparation of S/Ce-PABMS. (b) Schematic diagram of the mechanism of the S/Ce-PABMA for synergistic treatment of PD. (c) The cellular uptake of nanocarriers by SH-SY5Y cells assessed by confocal laser scanning microscope (CLSM) after 4 h incubation. Reproduced with permission [133]. Copyright 2022, WILEY-VCH Verlag GmbH & Co.

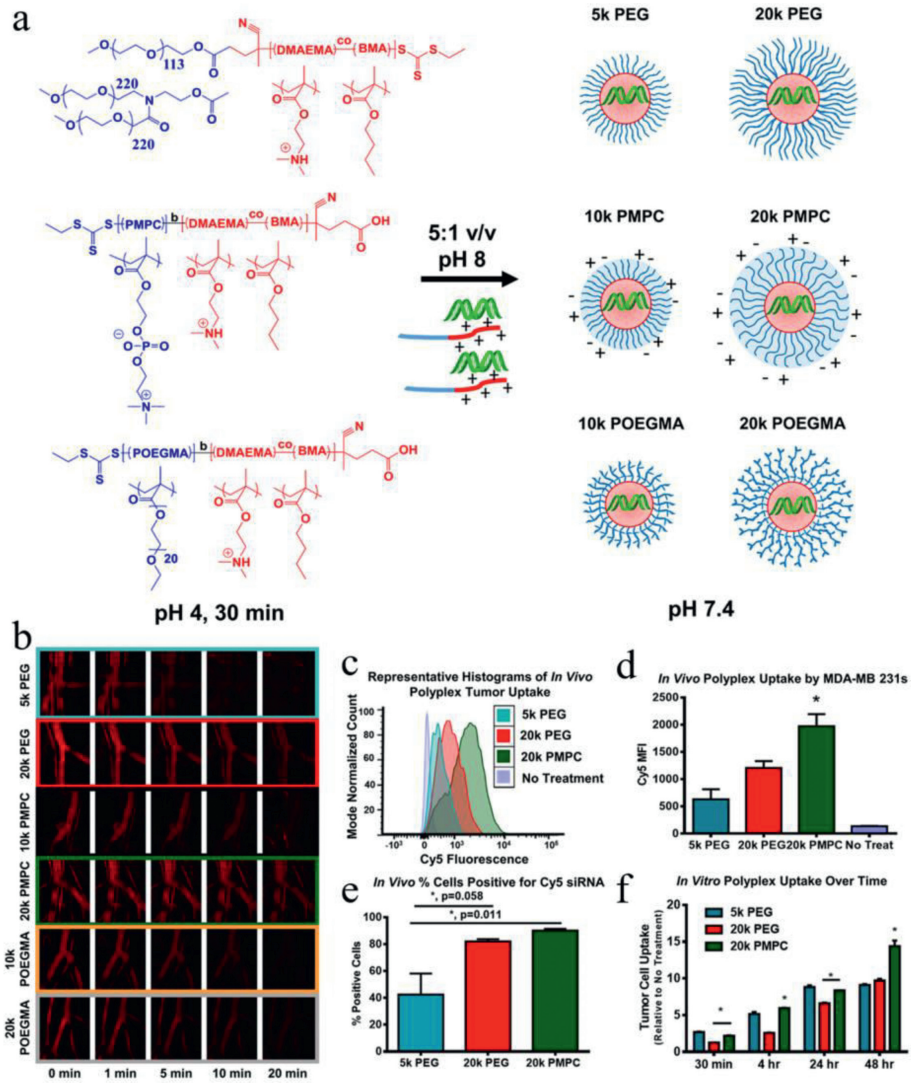

Fig. 6. (a) siRNA polyplexes containing varied corona architectures. (b) A panel of intravital microscopy images for visualization of pharmacokinetic differences between polyplexes shows an obvious increase in circulation time for 20k PEG and 20k PMPC compared to gold standard 5k PEG. (c) Representative histograms of polyplex uptake for tumor cells analyzed by flow cytometry from tumors harvested at 24 h post-treatment. (d) At 24 h, 20k PMPC polyplexes had significant, 63% increased mean Cy5 fluorescence in tumor cells compared to 20k PEG and 213% relative to 5k PEG. (e) 20k PMPC had significantly increased% positive cells at 24 h compared to 5k PEG, while 20k PEG had a strong tread toward increased% positive cells compared to 5k PEG. (f) In an *in vitro* time course, 20k PMPC polyplexes exhibited significantly higher uptake compared to PEGylated polyplexes. Reproduced with permission [138]. Copyright 2017, American Chemical Society.

jection of 1 mg/kg dose). In our previous studies, we showed that the nano-brushed chemical structure of zwitterionic PCB facilitated the cellular uptake of nanocarriers due to the enhanced topographic interaction between nanocarriers and cell membranes [6]. We observed the cell membrane structure of NSCs after treatment with nanocarriers modified with PEG and PCB *via* CLSM. The results showed that cells treated with PEGylated nanocarriers exhibited nearly spherical actin skeletons, whereas the cells displayed extended actin skeletons after incubation with nanocarriers with PCB. The filopodia enhanced the cellular uptake of PCB nanocarriers because of their active capturing ability. Therefore, zwitterionic polymers do not sacrifice, even promote the cellular uptake of nanocarriers.

Besides the shape effect, it has been demonstrated that surface charge of nanocarriers is a major determinant of cellular uptake [139]. Cationic nanocarriers enter the cells easily because of the adsorptive interactions with the negatively charged cell membrane [140]. However, cationic charges often induce opsonization, which results in a fast clearance from the blood circulation [141,142]. Therefore, it is desirable to develop zwitterionic polymer-based nanocarriers that are resistance to nonspecific protein adsorption in blood, but switch to positively charged to enhance cellular internalization.

Stimuli-responsive charge conversion strategy can achieve surface charge conversion from neutral to positive, resulting in improved cellular uptake, while maintaining the long blood circulation [143]. Some zwitterionic polymers have been found to have charge-transforming property, and their surface charges can be converted in response to pH through acid-responsive cleavage or protonation/deprotonation processes [144,145]. This strategy is suitable for cancer therapy as the pH of tumor tissue (6.5–6.8) is lower than that in blood and normal tissues [146]. As shown in Fig. 7, Wang *et al.* synthesized a polyprodrug that was composed of pH-responsive PCB-like zwitterionic segment and glutathione-responsive poly-camptothecin prodrug segment for tumor therapy [147]. The PCB-like zwitterionic structure consisted of positively charged quaternary ammonium and negatively charged carboxylate group formed by the reaction of amino group

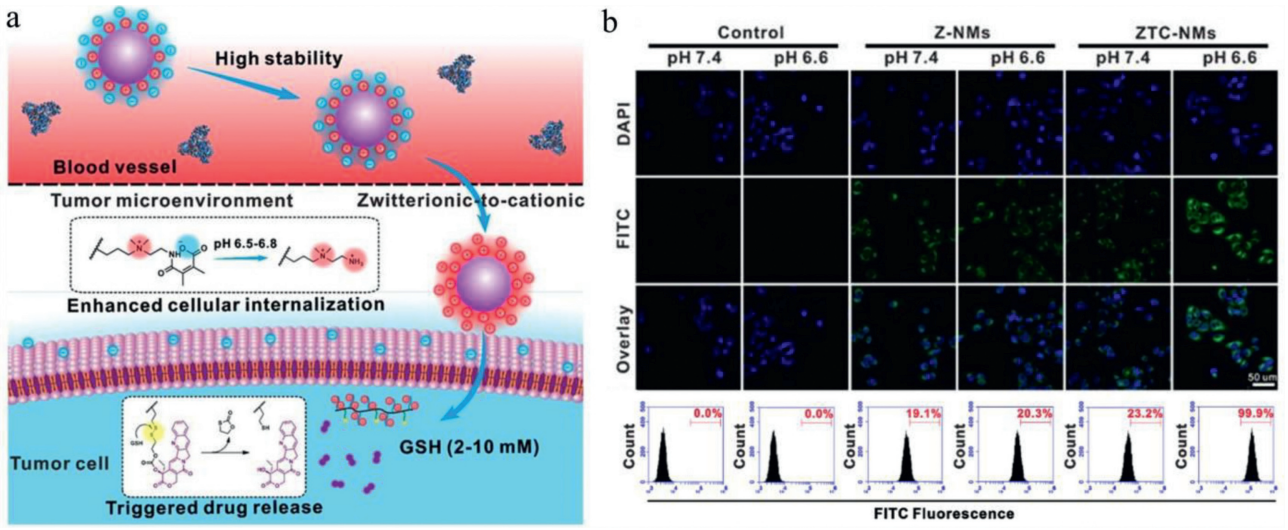

Fig. 7. (a) Schematic illustration showing the drug delivery process of ZTC-NMs. (b) Confocal fluorescence images and flow cytometry analysis of A549 cells upon incubation with different pH pretreated FITC-labeled Z-NMs and ZTC-NMs for 2 h. Reproduced with permission [147]. Copyright 2020, Ivyspring International Publisher.

and 2,3-dimethylmaleic anhydride (DMMA). The zwitterionic-to-cationic (ZTC)-based nanomedicine (ZTC-NMs) could realize prolonged blood circulation time, which facilitated tumor accumulation through the enhanced permeability and retention (EPR) effect. Compared with conventional zwitterionic surface, the amide bond formed between DMMA and amino group responded to tumor pH and achieved acid-responsive cleavage, resulting in strong cationic of highly positive quaternary ammonium salt. Obviously stronger green fluorescence was found inside the cells when incubated with pH 6.6 pretreated ZTC-NMs than pH 7.4 pretreated ZTC-NMs. Compared with Z-NMs with non-pH-responsive PCB-like zwitterionic segment, the ZTC-NMs showed the most potent antitumor effect on A549 tumor mice. The results indicated that the ZTC surface charge conversion ability of ZTC-NMs could enhance cellular uptake and therapeutic efficacy of drugs. Gao and co-workers prepared pH-sensitive zwitterionic dHAD by adjusting the *N*-deacylated degree of hyaluronic acid (HA) and grafting dodecylamine onto the dHA polymers for targeted and intracellular delivery of anticancer drug DOX [148]. The zeta potential of the negatively charged dHAD-DOX micelles was converted to the positive when the pH decreased to 5.4 and 6.2, respectively. The positive charges of the dHAD-DOX micelles could accelerate the cellular uptake through attractive interaction with the negatively charged cell membrane, which resulted in high antitumor efficacy, especially the dHAD-22-DOX micelles with higher degree of substitution of dodecylamine groups.

Additionally, enzyme-triggered charge reversal of zwitterionic polymers has also been applied to generate cationic charges once in the tumors for enhancing the cellular uptake and improving tumor penetration of nanocarriers [149–151]. Membrane γ-glutamyl transpeptidase (GGT), overexpressing on the external surface of endothelial cells and metabolically active tumor cells at the periphery of blood vessels, can cleave γ-glutamylamides with a structure-dependent activity [149]. An α-substituent on the γ-glutamylamide facilitates the hydrolysis. As shown in Fig. 7, Zhou and co-workers synthesized a GGT-responsive zwitterionic polymer-drug conjugate PBEAGA-CPT with a long blood circulation for tumor therapy [152]. 2-(L-γ-Glutamyl-L-α-amniobutyrylamino)ethyl acrylamide (BEAGA) was hydrolysed after incubation with GGT (10 U/mL), and the zeta potential of $PBEAGA_{18}$-CPT_5 became positive within 15 h. In comparison, $PEAGA_{18}$-CPT_5 with 2-(L-γ-glutamylamino)ethyl acrylamide (EAGA) did not change significantly and remained negatively charged even after 48 h. The resulting cationic conjugate undergone caveolae-mediated endocytosis and transcytosis, which enabled transendothelial and transcellular transport and a relatively uniform distribution throughout the tumor. The conjugates showed a potent antitumor activity in mouse models that led to the eradication of small solid tumors ($\sim$100 mm^3) and regression of large established tumor with clinically relevant sizes ($\sim$500 mm^3), and significantly extended the survival of orthotopic pancreatic tumor-bearing mice compared to that with the first-line chemotherapeutic drug gemcitabine. The GGT is overexpressed on the external surface of endothelial cells, and can enhance the transendothelial transport of drugs. In comparison, the physiological pH of perivascular compartment is neutral, where the pH-triggered charge reversal of zwitterionic polymers cannot achieve. Meanwhile, positively charged nanocarriers may disrupt the membrane structure and thus induce cell death. Therefore, it should be noted that it is important to keep a balance between the delivery efficiency and toxicity of these zwitterionic polymer-based nanocarriers with stimuli-responsive charge conversion ability.

3.4. Enhanced endosomal escape

The internalized nanocarriers are usually trapped inside the endosomes and ultimately end up in the lysosomes. Relative to normal physiological environment, the pH in the endosomes is about 5.5–6.5, and declines to approximately 4.0–5.0 in the lysosomes, where drugs would be enzymatically degraded and inactivated [153–157]. The degradation and inactivation of drugs would drastically decrease the therapeutic efficiency. It has been shown that the FDA-approved lipid NPs likely mediate only 1%–4% of encapsulated siRNA into the cytoplasm [158,159]. Therefore, it is vital for the nanocarriers to rapidly escape the endosomes, while maintaining stability in the blood circulation [160]. One approach to solve this problem is using the pH-dependent charge conversion strategy [161]. As mentioned above, some zwitterionic polymers can reverse their neutral charge to positive charge upon pH stimuli. This charge conversion could accelerate the endosomal escape by promoting the fusion with endosome membrane or proton sponge effect [162]. Therefore, zwitterionic polymers offer a path to overcome the challenge of endosome trapping.

Among zwitterionic polymers, PCB is highly resistant to nonspecific protein adsorption at pH 7.4, and the negatively charged carboxylate groups of the PCB could be protonated at low

pH, which makes it capable of enhancing endosomal escape of nanocarriers [163,164]. In our previous study, we modified DSPE lipid with PCB_{20} to construct cationic liposome/siRNA lipoplexes [165]. Compared with DSPE-PEG lipoplexes, the zeta potential of DSPE-PCB_{20} lipoplexes was 8.19 ± 0.53 mV at pH 7.4, and increased to 24.6 ± 0.87 mV when the pH value was decreased to 4.5. The CLSM images showed that the siRNA in DSPE-PCB_{20} lipoplexes had stronger endosomal escape than DSPE-PEG lipoplexes, which resulted in approximately 20% enhanced silencing efficiency of siRNA. In another study, the endosomal escape of nanocarriers with PCB was detected in the presence of bafilomycin A1 [58]. Bafilomycin A1 is a proton pump inhibitor, which selectively inhibits the vacuolar H^+-ATPase and prevents the acidification of endosomes/lysosomes. Compared with the NSCs without treatment with bafilomycin A1, bafilomycin 1 obviously inhibited the endosomal escape of FAM-let-7b antisense oligonucleotide. These results demonstrated that the protonation of PCB could enhance the endosomal escape of drugs. This phenomenon has been proved in other works [6,134,154].

4. Conclusion and further outlook

In general, an ideal delivery system is supposed to meet the following requirements including prolonged blood circulation time, specific tissue/cell targeting, enhanced cellular uptake and promoted endosomal escape, thereby improving the therapeutic efficacy and reducing the side effects. Although many nanocarriers have these functions, these nanocarriers often need complex components and multistep modification processes to achieve the above requirement. It is difficult for these complicated nanocarriers to get to the clinical translation stage. Therefore, it is intriguing to develop simple materials to achieve most of the requirements in the drug delivery process, which will be beneficial for the potential clinical application.

Due to the unique and diverse structures, one zwitterionic polymer would have multiple functions of targeting, enhanced cellular uptake and promoted endosomal escape beyond nonfouling. These properties render zwitterionic polymers an ideal class of materials to construct nanocarriers with simply component and structure. Among these zwitterionic polymers, PMPC might be the most widely used in drug delivery as it has been approved by FDA. However, it should be noted that PCB has many unique properties, such as easy preparation and modification, drug loading ability, excellent nonfouling properties, targeting to some tissues or cells such as BBB and intestinal epithelial cell layer, and enhanced endosomal escape *via* protonation. These properties are benefit for drug delivery. Therefore, PCB might be the best candidate material for drug delivery of nanocarriers. At the same time, more studies on PCB are needed to promote its clinical application. Furthermore, the existing zwitterionic polymers are difficult to solve all the issues in the process of drug delivery. Therefore, it is also necessary to develop more new zwitterionic polymers with universal functionality for drug delivery. Inspired from the discovery of PTMAO zwitterionic polymers [113], the molecules in nature would provide a source for the design of new zwitterionic polymers.

Besides the effect of overcoming the physiological barriers for nanocarriers, we speculate that some zwitterionic polymers might have bioactive functionality or even therapeutic effect on diseases due to their biomimetic structures. For instance, the monomer of PCB and PPC is the analogue of betaine and choline, respectively [166]. Betaine, a trimethylglycine, is widely distributed in animals. Betaine is an important methyl group donor and osmoprotectane [118]. In addition, accumulating evidence has shown that betaine has anti-inflammatory effect on some diseases such as diabetes, cancer and AD [167]. Choline is an essential nutrient, and choline and its derivatives serve as structural lipoproteins, blood and membrane lipids components [168,169]. Additionally, choline is a precursor of the neurotransmitter acetylcholine, and is close related with the neurodevelopment. With the similar structure, these zwitterionic polymers might have bioactive functionality and/or therapeutic effect on diseases, and this is needed for further study. In all, despite numerous efforts have been devoted in exploring the zwitterionic polymers for drug delivery, more studies are also required to successfully put the zwitterionic polymers in clinical field.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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