Article:
Lai, W.-F. orcid.org/0000-0003-0585-6396 (Accepted: 2025) Design and applications of polymersomes for oral drug administration. ACS Applied Materials & Interfaces. ISSN 1944-8244 (In Press)

Design and applications of polymersomes for oral drug administration

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Conflict of interest

The authors declare no conflict of interest.

Abstract

Polymersomes are nanostructures consisting of a hallow aqueous compartment enclosed by a coating of amphiphilic block copolymers, which undergo self-assembly during polymersome fabrication. Owing to the entangled nature of their membrane, polymersomes exhibit higher mechanical stability than some other extensively studied nanostructures such as liposomes. This also enables the properties of the polymersome membrane to be more easily tuned to meet practical needs, rendering polymersomes a potentially promising carrier to revolutionize the landscape of oral drug delivery research. Since the turn of the century, the use of polymersomes has been exploited in diverse areas, ranging from protein therapy to medical imaging. Yet, discussions exploring the opportunities and challenges of the development of polymersomes for oral drug administration have been scant. This article addresses this gap by offering a snapshot of the current advances in the design, fabrication and use of polymersomes as oral drug carriers. Various polymersome designs and preparation methods will also be discussed. It is hoped that this article cannot only highlight the practical potential of polymersomes in oral drug administration but can also shed light on the crucial challenges determining the wider clinical potential of polymersomes in the forthcoming decades.

Keywords

Polymersomes; block copolymers; colloidal behaviour; drug delivery; oral administration; controlled release; amphiphilicity

Introduction

Polymersomes (also known as polymeric vesicles) are nanostructures consisting of a hallow aqueous compartment enclosed by a coating of amphiphilic block copolymers, which undergo self-assembly during polymersome fabrication. [1-3] Compared with homopolymers and various types of copolymers (including random copolymers and alternate copolymers), block copolymers show unique tunability in structures and physical properties. [4] This makes fine-tuning of colloidal behaviour via changes in the chain length and in the structure of the block segment feasible. Owing to this feature, amphiphilic block copolymers are known to be able to form diverse types of particulate vehicles, ranging from worm-like micelles and micelles to polymersomes in an aqueous environment. [5-7] Over the years, various block copolymers [such as polyethylene glycol-*b*-poly(amino acid)s, [8] poly(ethylene glycol)-*b*-poly(ε-caprolactone), [9] and poly(ethylene glycol)-*b*-poly(*D*,*L*-lactide) [10]) have demonstrated the capacity of forming micelles via self-assembly. The generated micelles have successfully been adopted to deliver therapeutic agents. Recently, polymersomes generated by folate-conjugated pluronic P85/poly(lactide-co-glycolide) copolymer has been exploited for insulin delivery to fasting diabetic rats. [11] While no hypoglycemic effect has been observed in the group administered with free insulin, rats administered with the insulin-loaded polymersomes have exhibited significant and prolonged hypoglycemic effects. [11] This corroborates the clinical potential of polymersomes in pharmaceutical formulation.

Compared to many other carrier systems (*e.g.*, liposomes, micelles, and solid lipid nanoparticles), polymersomes show distinct advantages for mediating oral drug delivery. Their unique vesicular architecture, formed by the self-assembly of amphiphilic block copolymers, results in a bilayer membrane that is significantly thicker and more stable than that of liposomes. [12] This enhanced membrane robustness offers superior protection for encapsulated

drugs. Additionally, the physicochemical properties of polymersomes—such as size, surface charge, membrane permeability, and degradation rate—can be finely tuned through precise control of polymer composition and architecture. [13] This tunability enhances the ability of polymersomes to overcome the physiological and biochemical barriers associated with drug administration. Unlike many micellar systems, polymersomes are considered more stable and less prone to premature disassembly due to their kinetic stability. [14] This facilitates sustained drug release. Combined with their capacity to encapsulate both hydrophilic and hydrophobic drugs and their ease of surface functionalization, [15, 16] these features position polymersomes as a versatile and highly customizable platform with strong potential to overcome the multifaceted barriers of drug delivery.

Till now the use of polymersome-based carriers has already been exploited in diverse areas, ranging from protein therapy[17, 18] to medical imaging. [19-21] Despite this, most of the studies in the literature have exploited polymersomes mainly as carriers for systemic drug administration. [22-26] Efforts devoted to exploring the potential use of polymersomes as oral drug carriers have been scant. In fact, compared to parenteral routes (*e.g.*, intravenous, subcutaneous, and intramuscular routes), drug administration via the oral route has unique advantages ranging from non-invasiveness and convenience of operation to high patient compliance. Approximately 60% of commercially available small-molecule pharmaceutical products are administered via the oral route, [27] with around 90% of the global market share of all drug formulations intended for human use is estimated to taken up by oral formulation. [27] due to the presence of multiple barriers—ranging from the harsh gastric environment to metabolic breakdown of the drug in the intestinal region—unique to oral drug administration, achieving high efficiency of drug delivery via the oral route is more challenging than via other parenteral methods (**Table 1**). [28, 29] The objective of this article is to revisit the current status of the role of

polymersomes in oral drug administration. The latest advances in the design, fabrication, and optimization of polymersomes that have been used as oral drug carriers will also be reviewed. It is hoped that this article cannot only provide a snapshot of the development of polymersome-based carriers in oral drug delivery but can also offer insights into optimization and clinical translation of the developed carriers for future research.

Structural design of block copolymers and the polymersome thereof

Structures of block copolymers will play a vital role in determining the properties (including but not limited to including physical stability and membrane thickness) of the polymersomes generated. Such properties will in turn affect the drug encapsulation efficiency, release sustainability and metabolic fate of the polymersomes upon oral ingestion. To render the copolymer amphiphilic, both hydrophilic and hydrophobic blocks have to be incorporated into the structure of the copolymer. Poly(acrylates), poly(lactic acid), poly(caprolactone) (PCL) and poly(methacrylates) are some of the commonly used candidates for the hydrophobic block, though other polymers such as polydimethylsiloxane, poly(γ-benzyl-L-glutamate), polystyrene, poly(trymethylene carbonate) and poly(2-oxazoline) have been adopted in the literature. [30] For the hydrophilic block, poly(acrylic acid), polyacrylamides, poly(2-methyl-2-oxazoline), poly(amino acids) and poly(ethylene glycol) (PEG) are some of the polymers that have been extensively used in structural design of the amphiphilic block copolymer. [30]

When a block copolymer is designed for subsequent polymersome fabrication, one important factor to be considered is the molecular weight ratio of different blocks. The importance of this has previously been demonstrated in the case of poly(ethylene glycol)-*b*-poly(alkyl acrylate-co-methacrylic acid), in which manipulation of the composition of the ionizable polymer block has been found to alter the performance of the generated product in drug loading and pH-

dependent drug release. [31] Block copolymers with the molecular weight ratio of hydrophilic and hydrophobic blocks being 1:1 are, in general, thought to tend to self-assemble into micelles; whereas those with the ratio being 1:3 tend to form polymersomes. [32, 33] This, however, is only a general trend and various other factors (such as packing parameter and the volume fraction of each polymer block) could play a role. [34] For this, experimentation is often needed when an optimal molecular weight ratio of different blocks is sought for a particular block copolymer for polymersome fabrication.

In addition, right now most of the block copolymers designed for polymersome generation are electrically neutral. Incorporating charged blocks into a block copolymer is, however, one strategy to enhance the functionality of polymersomes through electrostatic interactions. The possibility of generating charged polymersomes has been demonstrated by one study, in which carboxyl groups [whose acid dissociation constant often lies in a range of 3-5, though its actual value could be affected by various factors (ranging from the temperature of the surrounding medium to the type of functional groups co-present in the same chemical entity) [35-39]] have been incorporated into poly(ethylene glycol)-poly(caprolactone-gradient-trimethylene carbonate) (PEG-p(CL-g-TMC)) amphiphilic block copolymers. [40] The rationale of this structural design is based on the understanding that, a block copolymer, and the polymersomes thereof, preferably shows high stability at gastric pH (1.5-2) and be able to disassemble at intestinal pH (6-7.4) if it is to be used as an oral drug carrier. [40] The polymersomes generated from the copolymers have been found to remain intact in pH of 5.0 or below, but when the pH of the surrounding medium has been increased to 6.5, deprotonation of the carboxyl groups has occurred, leading to a remarkable increase in hydrodynamic radius and polydispersity. [40] Such changes have led to pH-dependent alterations in mean square displacement and diffusion coefficient exhibited by the polymersomes. [40] In fact, over the years, charged polymersomes

have already been adopted to manipulate the colloidal stability of polymersomes in different media[41] and to attain on-demand drug release from drug-loaded polymersomes. [42] Recently, fabrication of charged polymersomes has been facilitated by advances in microfluidic technologies, with which polymersomes have been successfully generated from polyacrylic acid-block-polystyrene (PAA-*b*-PS) by using a flow approach. [43] The device for continuous flow polymersome formation enables not only optimization of the self-assembly conditions but also in-line dialysis for removal of organic solvents.

Strategies to generate polymersomes for oral drug administration

Amphiphilic block copolymers can undergo self-assembly in an aqueous environment to form nanostructures. Such process is driven predominantly by the tendency of the block copolymers to attain the lowest total free energy of the system ($\Delta G < 0$). [44, 45] This is achieved by minimizing, at the expense of the entropy of the single chains, the enthalpy gain caused by hydrophobe-water interactions. The preferentially adopted morphology of the generated self-assembled nanostructures can be predicted by using a dimensionless "packing parameter" (denoted as p) which can be calculated by using the following equation (Eq. 1):

$$p = \frac{v}{a_0 l} \quad (1)$$

where v is the volume of the hydrophobic chains, a_o is the contact area of the head group, and l is the length of the hydrophobic tail. In general, when p is less than 1/3, formation of spherical micelles is favoured during the self-assembly process. The micelles are expected to adopt a cylindrical shape when p is between 1/3 and 1/2. When p is further increased to be between 1/2 and 1, formation of polymersomes is favoured (**Figure 1**).

Encapsulation of drugs within polymersomes can be achieved in two ways (**Figure 2**). The first method involves generating polymersomes, followed by electroporation and extrusion to load drug molecules inside.[46] This method offers flexibility in selecting drug molecules to load after the self-assembly process, but it is limited to hydrophilic drugs and requires multiple stages. The second method involves mixing drug molecules with amphiphilic block copolymers, allowing the drug to be encapsulated during polymersome formation.[46] This single-step method enables the loading of both hydrophobic and hydrophilic drugs, and it is more commonly used. One extensively used procedure to generate drug-loaded polymersomes is solvent evaporation. This approach has previously been adopted to generate polymersomes from FA-P85-PLGA for oral administration of insulin. During polymersome preparation, a tetrahydrofuran solution of FA-P85-PLGA is first added to an aqueous solution of insulin, followed by constant stirring of the generated emulsion.[11] Upon evaporation of organic solvent, the generated insulin-loaded polymersomes are retrieved by centrifugation before dispersion into water for subsequent use.[11] Apart from evaporation of the organic solvent from an emulsion to generate polymersomes, some polymersomes could be produced and retrieved by taking advantage of the variations in solubility in different solvents. The use of this method can be exemplified in a recent study,[47] in which a nanogel-polymersomes system [consisting of chitosan diacetate (CDA), methoxy-poly(ethylene glycol)-*b*-poly(lactide) (MPP), and D-α-tocopheryl polyethylene glycol succinate (TPGS)] with permeation-glycoprotein inhibition capability has been developed for co-delivery of oxaliplatin and rapamycin for chemotherapy.[47] The polymersomes are generated via solvent switch, in which a DMSO solution containing MPP and the two drugs is added dropwise into an aqueous solution of TPGS, followed by constant stirring and subsequently dialysis against deionised water.[47] The generated polymersomes (namely TMOR) are then modified by nanoparticles generated from CDA,

forming TMOR-CDAN, to prolong the residence time (and to prevent degradation) of the loaded drugs in the gastrointestinal tract. [47]

Apart from the methods mentioned above, polymersome-based oral drug carriers can be prepared by thin-film rehydration, [48] sonication[49] and direct dissolution. [50] Some of these methods have already been reviewed elsewhere. [51, 52] Recently, fabrication of polymersomes has benefited from advances in microfluidic technologies, which enable attainment of single droplets and multiple droplet arrays with precisely controlled composition and narrow size distribution. In a Y-shaped microfluidic device with toroidal mixer generated by both photo-lithography and soft-lithography, a DMSO solution of the polyvinyl alcohol-polyethylene glycol-graft-copolymer is mixed with deionized water (**Figure 3**). [53] In order to minimize the free energy involved, [54] the copolymer undergoes self-assembly, generating polymersomes for co-delivery of nisin and curcumin. [53] Although the use of microfluidics in polymersome generation is still not as prevalent as conventional methods (*e.g.*, solvent switch and evaporation), over the last several decades microfluidics has emerged as a compelling technology enabling generation of single droplets and multiple droplet arrays with precisely controlled composition and size distribution. [55-58] Till now microfluidic technologies have already been applied to diverse areas, ranging from liposome production[59-62] to generation of metal nanoparticles. [63, 64] Their tract record of application in fabricating nanoparticulate drug delivery systems, along with their potential to enable automation miniaturization and their capacity of manipulating fluids at a small length scale, are envisaged to contribute to the increasing use of microfluidic technologies in polymersome fabrication and optimization in the upcoming decade.

Roles and use of polymersomes in oral drug administration

The oral route is one of the most preferred routes of drug administration because not only of its ease of operation but also of its non-invasiveness and hence high patient compliance. However, it is not without reason that from time to time the parenteral route, rather than the oral one, is adopted. This is because the efficiency of drugs administered orally is easily impended by biological and biochemical barriers imposed by the gastrointestinal tract. [65-68] Examples of biological barriers include the low pH of the gastric environment and the mucus membrane lining the gastrointestinal tract. [69, 70] Biochemical barriers comprise intestinal metabolism (mediated by digestive enzymes), brush-border metabolism (facilitated by enzymes located on the microvilli of enterocytes), and intracellular metabolism (occurring within enterocytes, involving enzymes such as cytochrome P450 and phase II conjugating enzymes). [71, 72] The first-pass effect— referring to the presystemic metabolism of a drug in the intestine and, more significantly, in the liver after absorption and transport via the hepatic portal vein—can also reduce the observed oral bioavailability. In addition, properties of the drug *per se* will significantly influence oral bioavailability. In general, drugs that are classified by the Biopharmaceutical Classification System (BCS) as Class I are ideal for administration via the oral route because these drugs show high solubility and permeability. [73] On the other hand, the oral bioavailability of BCS Class II, III and IV drugs may not be satisfactory because these drugs exhibit poor solubility and/or poor permeability. [74, 75] Major roles of polymersomes in oral drug administration are, therefore, either to assist the delivered agent to overcome some of the aforementioned barriers or to modify properties of the delivered agent to enhance oral bioavailability (**Figure 4**).

Enhancing drug stability in the gastrointestinal tract

One major role of polymersome-based oral drug carriers is to enhance the stability of the delivered agent in the gastrointestinal tract. The technical viability has been demonstrated by

the case of rapamycin, which generally undergoes degradation via ring opening readily under an acidic environment. [76] The poor stability of this drug makes it highly susceptibility to gastric action upon oral administration, leading to low oral bioavailability. [76] A previous study has demonstrated that more than 90% of free rapamycin has undergone degradation after being incubated at pH 1.2 for 90 min. [47] Yet, after encapsulation of rapamycin into polymersomes, only 20% of rapamycin has been degraded. [47] A similar observation of the role of polymersomes in enhancing drug stability has been made on insulin, which is a protein and hence is susceptible to denaturation under the gastric environment. In pepsin-containing simulated gastric fluid (pH 1.2), over 85% of insulin in a free insulin solution has been degraded; whereas only around 35% of insulin encapsulated by FA-P85-PLGA polymersomes has undergone degradation. [11] Furthermore, in trypsin-containing simulated intestinal fluid (pH 6.8), only less than 15% of insulin in a free insulin solution has been maintained; however, after encapsulation by the polymersomes, the percentage of insulin that has been protected from degradation has reached as high as 76%.[11] All these corroborate the role of polymersomes in protecting vulnerable drugs from degradation after oral administration.

Apart from the fragile drugs which are readily degradable, polymersomes can stabilize drugs that are susceptible to metabolism after oral ingestion. This is evidenced by the case of sorafenib, which is known not only to display poor solubility in a wide range of pH values (1.2–7.4) [77, 78] but also to be highly susceptible to first-pass metabolism, thereby having poor oral bioavailability. [79-81] In an earlier study, polymersomes generated from poly(butadiene)-block-poly(ethylene oxide) (PB-*b*-PEO) have been used as carriers of sorafenib. [82] Compared with mice given a sorafenib suspension via the oral route, those orally administered with sorafenib-loaded PB-*b*-PEO polymersomes have been found to have a higher plasma drug concentration and a higher C_{max} value. This reveals the success of the polymersome-based

carrier in protecting the delivered drugs from first-pass metabolism upon oral administration. Although the exact mechanism adopted by polymersomes to achieve this has yet to be fully elucidated, it has been reported that polymeric micelles with appropriate design could redirect the absorption pathway of the encapsulated drug from the portal circulation to the intestinal lymphatic system so as to bypass the first-pass effect in the liver. [83] Furthermore, polymersomes could be engineered to enhance cellular uptake via mechanisms such as transcytosis, [84] particularly through M-cells in Peyer's patches, which may facilitate absorption routes less exposed to hepatic metabolism. [85] Together with the fact that polymersomes could provide a protective barrier that shields encapsulated drugs from enzymatic degradation in the gastrointestinal tract, thereby increasing the likelihood that the active drug reaches systemic circulation intact, [2, 86] all these features may help explain the ability of polymersomes to enhance the oral bioavailability of drugs susceptible to first-pass metabolism.

Facilitating intestinal absorption and cellular internalization

Apart from enhancing the oral bioavailability of delivered drugs by improving drug stability, polymersome-based carriers may proactively facilitate intestinal absorption and cellular internalization of orally-administered agents. The viability of using polymersome-based carriers to enhance cellular uptake of orally-administered agents is partially evidenced by poloxamer 401 polymersomes, which have been adopted for oral delivery of proteinaceous agents. [87] In the epithelial/macrophage co-culture model, adalimumab-loaded poloxamer 401 polymersomes have shown the ability to reduce the proinflammatory cytokine level, with the detected concentration of tumour necrosis factor α (TNF-α) being negatively related to the concentration of adalimumab loaded into the polymersomes. [87] Furthermore, immunoglobulin G (IgG) delivered by the polymersomes has led to intestinal epithelial permeation in Caco-2 cell monolayers 2.7-fold more effective than unencapsulated IgG. [87] To elucidate the possible

cellular uptake mechanism adopted by polymersome-based carriers, an earlier study has treated Caco-2 cells with chlorpromazine (to disrupt the assembly and disassembly of clathrin), filipin (to disrupt caveolae structure by binding to cholesterol), and colchicine (to lead to the disassembly of microtubules). [11] Upon cell treatment, cellular uptake of polymersomes has been found to be inhibited. [11] This reveals that cellular internalisation of the polymersomes could be mediated concomitantly by micropinocytosis, clathrin-mediated endocytosis and caveolae-mediated endocytosis.

Apart from enhancing cellular internalization, polymersome-based carriers can modulate the absorption profile of the delivered agent in the gastrointestinal tract. This has been demonstrated by the pH-responsive PEG-p(CL-*g*-TMC) polymersomes developed recently for oral administration of mycophenolate mofetil. [40] Mycophenolate mofetil is a drug used as an alternative therapy for patients with inflammatory bowel disease unresponsive to conventional treatments. [88] Its feasibility to be delivered via the oral route has been impeded by its low solubility in the small intestine and its high solubility (and absorption) in the stomach. The aim of delivering the drug using those polymersomes is therefore to reduce drug absorption in the stomach and to increase absorption in the small intestine. Upon oral administration of mycophenolate mofetil-loaded polymersomes to male Wistar Han rats that have been underwent a 12-hour fasting period, a higher amount of the loaded drug has successfully reached the intestinal region even though absorption in stomach has still been observed. [40]

Ameliorating adverse effects brought about by the administered drug

The technical feasibility of ameliorating adverse effects brought about by administered drugs has been revealed by Dickson Pius and coworkers, [47] who have apply nanogel-modified polymersomes to co-deliver of oxaliplatin and rapamycin for synergistic chemotherapy. In the

in vivo context, the effectiveness of the system in mediating chemotherapy via the oral route has been confirmed by using the 4T1 subcutaneous carcinoma model, which has been established by infiltrating mice with murine mammary carcinoma 4T1 cells into the left axilla. [47] Comparing with using free drugs or drug-loaded TMOR polymersomes, reduction of the tumour size has been found to be more significant in the group treated with drug-loaded TMOR-CDAN. [47] Importantly, the colon length of the treated mice has been examined to determine the severity of drug-induced inflammation caused by the treatment. [47] Compared with those treated with free drugs, those treated with drug-loaded TMOR and TMOR-CDAN have been found to undergo less significance of colon shortening. [47] This reveals that the polymersomes have played a role in reducing chemotherapy-induced gastrointestinal toxicity.

This amelioration of adverse effects can be attributed to the ability of polymersomes to offer controlled or sustained drug release, minimizing sudden spikes in systemic drug concentration that can trigger toxicity. The co-encapsulation of drugs also allows for synergistic action at lower doses, potentially reducing the need for high concentrations of each agent and thereby limiting side effects. Apart from these, polymersomes can shield sensitive cell membranes from direct contact with cytotoxic drugs to improve the safety profile of the administered agents. This has been confirmed in an earlier study, [82] which has treated human erythrocytes with a suspension of sorafenib (200 μg/mL) and has found that around 9% of the treated cells undergo haemolysis. On the other hand, upon encapsulation by PB-*b*-PEO polymersomes, the percentage of haemolysis has been found to be negligible. [82] Altogether, the role of polymersome-based carriers in mitigating adverse effects of orally administered agents results from their combined ability to modulate drug release, lower the effective dose, and limit cellular exposure to cytotoxic drugs.

Optimization for enhanced performance in oral drug delivery

Performance of polymersomes in oral drug delivery is affected largely by the structure of the amphiphilic block copolymers as well as the properties of the generated polymersomes. For this, optimization of the delivery efficiency mediated by polymersome-based oral drug carriers is generally conducted in these aspects. In the following section, major strategies to enhance the design and preparation of polymersomes will be discussed for oral drug administration.

Manipulation of structural properties of block copolymers

Polymersomes are generated from self-assembly of amphiphilic block copolymers. Changing the structure of the copolymer will lead to alteration in the self-assembly process and the structure of the generated nanoparticulate systems. This has been demonstrated by the case of the PEG-PLA copolymer. By fixing the molecular weight of PEG to be 5kDa and by manipulating the block length of PLA, the copolymer has been found to form micelles when the molecular weight of the PLA block is 5kDa and to form polymersomes as the molecular weight of the PLA block increases to 15kDa (**Figure 5**). [5] This is largely due to the bulkiness of the hydrophobic PLA segment, making it fail to fit in the interior of a micelle and hence forming a bilayer sheet instead. [5] In addition, altering the molecular weight of hydrophobic segments could lead to changes in structural features (particularly the membrane thickness) of the generated polymersomes. As polymersomes have a structure consisting of an aqueous core, along with a hydrophobic membrane and hydrophilic corona, increasing the membrane thickness of polymersomes has been found to facilitate hydrophobic agents to be loaded into the polymersomes. This has been shown feasible in previous studies, in which polymersomes have been used to deliver paclitaxel[89] and sorafenib. [82]

Furthermore, to enhance controlled release of the orally-administered drug, various functionalities sensitive to pH, redox conditions, or various physiological factors could be incorporated into the drug delivery system. This approach has been adopted in various types of carriers, ranging from metal-organic frameworks[90] and composite gels[91, 92] to polymeric nanoparticles. [93, 94] In terms of polymersomes, this approach can be adopted by incorporating the respective functionalities into block copolymers before polymersome fabrication. The possible use of this approach has been partially demonstrated by the case of polymersomes generated from PEG-p(CL-*g*-TMC), in which carboxyl groups have been added to render the subsequently generated polymersomes pH-responsive, for oral delivery of immunosuppressants. [40] Release of the loaded drug from the polymersomes has been found to be initiated when the pH of the surrounding medium reaches 6.5 (pH of the duodenum) or 7.5 (pH of the small intestine), with 90% of the loaded drug being released within the first two hours. [40] The release profile fits well with the Ritger-Peppas model and follows non-Fickian diffusion. [40] The success of achieving controlled release in the temporospatial sense can enhance the oral bioavailability of the delivered drug, which is released only when the carrier reaches the desired site of action.

Optimization of preparation conditions

To optimize the performance of polymersome-based oral drug carriers for preclinical and clinical translation, the self-assembly conditions have to be properly controlled during preparation of polymersomes because they could significantly influence the structure of the generated self-assembled systems. This has been revealed by a recent study, in which PAA-*b*-PS polymersomes have been generated using a flow self-assembly setup. [43] In the setup, a stream consisting of PAA-b-PS in tetrahydrofuran is co-flow with a stream consisting of hydrochloric acid (HCl) (which on one hand can modulate the charged state of the PAA blocks

and on the other hand, can induce self-assembly of the polymersomes via its non-solvent nature relative to PS) (**Figure 6**). Results showed that changing either the concentration of HCl or the content of tetrahydrofuran could lead to different self-assembled structures (micelles, polymersomes, and solid particles) being generated. In brief, when the concentration of HCl is low, the PAA blocks of PAA-*b*-PS tend to be deprotonated. This results in charge repulsion, leading to the formation of a comparatively high hydrophilic volume fraction, favouring micelle formation. On the other hand, if the concentration of HCl is too high, the PAA blocks of PAA-*b*-PS will be fully protonated. This results in an increase in the hydrophobic volume fraction, favouring the formation of particles deficient of apparent membrane or internal structures. Here it is worth noting that the optimal conditions of polymersome preparation may vary not only from one block copolymer to another but also from one application to another. For this, the preparation conditions should be optimized based on the characteristics of the specific amphiphilic block copolymer and the need of the specific application. This has been partly evidenced in the case of PB-*b*-PEO, in which the critical aggregate concentration of a block polymer for polymersome formation has been found to be affected by the molecular weight of the copolymer. [82] The optimal concentration of the copolymer concentration during polymersome preparation, therefore, has to be determined in a case-by-case manner.

Refinement of physicochemical properties of polymersomes

Once polymersomes are generated, their physicochemical features (ranging from size and surface properties to morphology) could remarkably influence their performance in oral drug administration. From a physiological perspective, the mucus layer, with its mesh-like network and brush-like architecture, functions as a size-selective barrier that restricts the movement of larger molecules. [95, 96] Particles generally need to be smaller than 200 nm in order to effectively penetrate the mucus layer. [97] As far as the size of polymersomes is concerned, it is worth noting

that the size of plain polymersomes may not effectively predict the pharmacokinetic profile exhibited by the drug-loaded ones upon oral injection. This is owing to the fact that the size of the polymersomes could be changed upon drug loading. The possibility of this has been demonstrated by the case of PEG-p(CL-*g*-TMC) polymersomes, whose hydrodynamic diameter increases from 90.8 ± 1.2 nm to 106.7 ± 1.9 nm upon drug encapsulation. [40] In addition, changing the amount of a loaded drug could significantly alter the hydrodynamic diameter of the generated polymersome, leading to changes in the pharmacokinetic profile. This has been reported by Dickson Pius and coworkers, [47] who have found that by changing the amount of oxaliplatin loaded into polymersomes from 1-10 mg, the size of the generated polymersomes changes from over 300 nm to around 155 nm and back to over 300 nm again. For this, characterizing the size of the polymersomes should be done after the drug loading process, with the identity and amount of the loaded drug being known at the time of size determination.

Not only the size but also the zeta potential of a carrier can influence the efficiency of oral drug delivery. Negatively charged and neutral particles, in general, penetrate the mucus layer more easily. [98] In contrast, positively charged particles exhibit lower mobility in mucus but greater cellular uptake via endocytosis than their negatively charged counterparts. [99, 100] Controlling the zeta potential is, therefore, crucial in the design of polymersomes. Yet, it is important to note that zeta potential of polymersomes may change during the process of drug loading. This has been hinted at by the case of the self-assembled carrier formed by the PLGA-PEG-PLGA copolymer. While increasing the concentration of the loaded US597 from 3 mg/mL to 30 mg/mL has been found to have minimal effect on the encapsulation efficiency and loading efficiency, [101] an increase in zeta potential from 5.76 ± 1.1mV to 10.65 ± 1.5 mV has been observed. Such changes may be due to the coating of the self-assembled carrier with US597.

[101] During the drug loading process, while the hydrophobic PLGA blocks in the core interacts with the lipophilic rigid triterpenoids ring structure of US597, the hydrophilic PEG blocks on the surface of the self-assembled structure also interacts with the polar NH_2 group of the drug. Such polymer-drug interactions lead to changes in the zeta potential of the drug-loaded carrier. Here it is worth that when the drug to be delivered possesses both hydrophilic and lipophilic groups, care should be taken in carrier design to avoid initial burst release. Taking US597-loaded carrier formed by self-assembly of the PLGA-PEG-PLGA copolymer as an example. Due to the rapid dissociation of surface-bounded drug molecules, a significant initial burst release has been observed. [101] The release profile turns to be sustained and steady only after 40 hours, in which release of drug molecules entrapped inside the self-assembled structure becomes dominant. [101]

Apart from optimizing the size and zeta potential, surface modification can help to enhance the efficiency of polymersomes in oral drug administration. This has been shown by the case of PLGA-P85-PLGA polymersomes. Upon oral administration of the insulin-loaded polymersomes to fasting diabetes rats, blood glucose depression of 25.3% at 2 h and 43.7% at 4 h has been observed. [11] However, upon modification of the surface of the insulin-loaded polymersomes with folate, blood glucose depression achieved has been increased to 36.8% at 2 h and 59.3% at 4 h. [11] In addition, compared with the AUC value of the PLGA-P85-PLGA polymersomes (211 ± 19.7 μ IU h/ml), that of folate-incorporated polymersomes has been reported to be 1.27-fold higher, [11] leading to a substantially higher plasma insulin concentration (27.6 ± 3.67 μ IU/ml for PLGA-P85-PLGA polymersomes vs. 35.8 ± 5.27 μ IU/ml for the folate-incorporated ones) 6 hours after oral administration to diabetes rats. [11] Besides incorporation of ligands, polymersome surface could be modified by morphologically. The technical feasibility of this has been demonstrated by Thomas and coworkers, [102] who have

adopted nucleobase pairing to direct the formation and lengthening of nodes on the outer surface of polymersomes. By adding a short diblock copolymer possessing complementary thymine side chains onto the surface of the polymersomes, an increase in steric crowding at the hydrophilic/hydrophobic interface is resulted. [102] Such steric crowding subsequently initiates node formation and elongation (**Figure 7**). [102] Once the morphology of the polymersome surface can be fine-tuned, it is anticipated that the pharmacokinetic profiles of polymersomes could be better tailored to meet different needs of oral drug administration.

Last but not least, the shape of polymersomes could be manipulated, too. Changing the shape of polymersomes cannot only alter drug release kinetics by modifying both the surface-to-volume ratio and aspect ratio of the system, [103-105] but also influences both their interactions with intestinal cell surfaces and the degree of mucosal entrapment they experience. [105, 106] The shape of polymersomes is, therefore, an important consideration in the design of carriers for oral drug delivery. [107] It can be manipulated by altering the structure of the block copolymer. This has been shown by the polymersomes generated by using an amphiphilic copolymer consisting of hydrophilic poly(ethylene glycol) (PEG) and hydrophobic poly(trimethylene carbonate-azobenzene) [P(TMC-AZO)]. [108] The polymerization degree of P(TMC-AZO) has been found to determine the morphology of the self-assembled structures. By having the number of monomers in the P(TMC-AZO) chain to be 12, small micelles with a diameter of around 20 nm are formed upon self-assembly of the copolymer. [108] Increasing the number of monomers in the chain to 20-25 causes the generated micelles to be a larger size and get connected. [108] A further increase in the length of the P(TMC-AZO) chain leads to the formation of ellipsoid-like vesicular nanostructures. [108] When the number of monomers in the P(TMC-AZO) chain increases to 45, tubular polymersomes are obtained. [108] The generated tubular polymersomes exhibited photo-responsive behaviour upon UV/Vis light irradiation, and can be

transformed to linear micelles upon light stimulation. [108] Although the polymersomes have not yet been tested for oral drug delivery, but the technical feasibility of manipulating morphological features of polymersome-based oral drug carriers has been corroborated.

Opportunities and challenges

As far as oral drug administration is concerned, polymersomes have so far been used only as discrete nanoparticulate systems for drug delivery in the literature. In fact, polymersomes have the potential to serve as colloidal building blocks to generate higher-order clustered structures. This can be achieved by using not only DNA base-pairing interactions to bind polymersomes with other colloidal components[109-111] but also electrostatic interactions. The feasibility of the latter has been demonstrated by a recent study, [112] in which positively charged polymersomes have been used as core particles on which negatively charged micelles can electrostatically attach to them as satellite particles (**Figure 8**). The positive charge of the polymersomes and the negative charge of the micelles come from the presence of poly(acrylic acid) (PAA) and quaternized poly(4-vinylpyridine), respectively, in their structures. Such an approach of clustering enables the build-up of higher-order structures from polymersomes regardless of the degree of fluidity of the polymersome membrane. Examining the impact of variations in the hierarchical structures generated by polymersomes on the pharmacokinetic profile of the loaded drug upon oral administration will potentially increase our understanding of carrier design and be one of the promising directions for future research.

Modification of polymersomes with other nanoparticulate systems is another area that is worth paying attention to in future research because this can enhance modulate the functionality of polymersomes in oral drug delivery. For example, by incorporating a near-infrared fluorescent dye and a paramagnetic probe [*viz.*, gadolinium (Gd(III)) cations] into polymersomes generated

from poly(acrylic acid-co-distearin acrylate), the polymersomes show potential to be used as a diagnostic tool for magnetic resonance (MR) imaging and near-infrared imaging. [113] More recently, a study has incorporated polymeric nanoparticles into polymersomes and has successfully enhance the delivery efficiency to the intestinal region. [47] When plain polymersomes are used at pH 1.2, 84% of loaded oxaliplatin and 36% of loaded rapamycin have been released within the first 2 hours; however, after modifying the polymersomes with polymeric nanoparticles, less than 12% of the loaded drugs have been released in the simulated gastric conditions. [47] This implies that a large percentage of the loaded drugs could be delivered to the intestines and corroborates the feasibility of modulating oral drug delivery performance by merely incorporating external nanoparticles into the polymersome-based carrier.

Here it is worth mentioning that while the effect of polymersomes in increasing the percentage of orally administered drugs to reach the intestinal region has been widely demonstrated in the literature, possible retention of polymersomes in the stomach due to the mucoadhesive properties of the block copolymer should not be overlooked and it may reduce, rather than increase, the overall oral bioavailability of the delivered agent. [40] This concern has been raised by Tollemeto and coworkers, [40] who have used a technique based on quartz crystal microbalance with dissipation (QCM-D) to confirm mucosal retention of polymersomes. Characterizing mucosal retention has been technically challenging outside the body, but light has been shed recently by Hearnden and coworkers, [114] who have first seeded primary oral keratinocytes and oral fibroblasts onto de-epithelized dermis (DED), following by raising the cell-attached DED to an air-liquid interface to facilitate the occurrence of epithelial stratification. [114] Upon incorporation with confocal laser scanning microscopy (CLSM), the penetrating capacity of rhodamine-labelled polymersomes in the 3D tissue-engineered oral mucosa has been successfully determined. [114] Such technique makes *ex vivo* evaluation of the

penetration and retention of polymersomes in a mucosal membrane technically feasible. A similar approach has also been reported for investigating penetration of many other nanostructures administered via diverse routes of administration. [115-117] The penetration and retention of polymersomes upon oral administration is worth exploration in upcoming studies when their performance in oral drug delivery is examined. [118]

Finally, although numerous polymersomes have been developed and tested since the turn of the last century, the transition from laboratory research to clinical trials has yet to be successfully achieved. One major barrier to clinical development lies in the complexity of polymersome formulation and the lack of scalable manufacturing methods. The synthesis of block copolymers often requires multiple steps, [119] making it difficult to achieve batch-to-batch consistency at industrial scale. The lack of manufacturing standardization is another factor posing challenges for regulatory approval and commercial viability during the clinical translation of polymersome research. To overcome this issue, future research should focus on developing simpler, aqueous-based or solvent-free synthetic routes that are scalable and reproducible. Furthermore, although many studies have reported promising in vitro and small animal model results, [120-124] few have extended these findings to large animal models or clinically relevant disease systems, not to mention elucidating the long-term pharmacokinetics of polymersomes. Given that some polymersomes are constructed from non-biodegradable or partially degradable polymers such as poly(ethylene oxide)-*b*-poly(butadiene) [125-127] or poly(styrene)-based blocks, [128-130] they may accumulate in tissues and induce long-term toxicity. Exploring the use of fully biodegradable and biocompatible polymers in polymersome design, as well as incorporating stimuli-responsive linkages that degrade under physiological conditions, would be a promising direction for future research.

Regulatory challenges present an obstacle to clinical translation, too. Although polymersomes have been studied for decades, currently there are no approved products or established regulatory precedents that could serve as benchmarks. This creates uncertainty around the requirements for preclinical data and safety assessments. Owing to unclear regulatory pathways and uncertain market returns, pharmaceutical companies are generally hesitant to invest in polymersome-based technologies. This hesitancy further constrains the clinical development of polymersome-mediated oral drug delivery. Addressing this problem will require proactive engagement with regulatory agencies to define acceptable parameters for clinical progression. Collaborative efforts among researchers, industry partners, and regulatory bodies will be essential to create a supportive framework for the clinical evaluation of polymersomes.

Concluding remarks

Polymersomes, as self-assembled nanostructures generated from amphiphilic block copolymers, exhibit high biocompatibility, excellent stability, and remarkable tunability in properties, making them promising candidates for drug administration, including oral drug delivery. As detailed in the sections above, the application potential of polymersomes as oral drug carriers has been supported in the literature. Increasing understanding of self-assembly kinetics, as well as factors influencing the pharmacokinetic profiles of orally administered agents, has facilitated the design and optimization of polymersome-based oral drug carriers. With ongoing advances in artificial intelligence and molecular modelling, not only the elucidation of possible interactions (in forms of fusion and fission) among polymersomes upon oral ingestion but also the possibility of merging multiple block copolymers in polymersome fabrication (as well as how the polymer blend approach affects the underlying self-assembly process) are expected to be streamlined over the upcoming decade by computational simulations, including coarse-grained simulation (in which a cluster of atoms is combined into

one interaction particle, enabling modelling of complex polymeric systems), which allows the molecular details underlying the mechanism and behaviour of polymersome formation to be studied in a way that can hardly be achieved experimentally. Along with the increasing sophistication of the design and optimization of polymersomes, the role of polymersomes played in oral drug administration is envisaged to be increasingly prominent in the coming years.

Despite the promising potential of polymersomes in oral drug delivery, further research is required to fine-tune the hydrophilic/hydrophobic block ratio in amphiphilic block copolymers for polymersome fabrication to enhance in vivo stability and optimize drug release kinetics. Achieving controlled release at precise locations in the gastrointestinal tract is essential, with stimuli-responsive block copolymers offering a potential solution. However, consistent and predictable release profiles across different physiological conditions still need to be established. Additionally, the ability to scale up production from laboratory to industrial scale is a critical challenge. Current methods of polymersome fabrication struggle with maintaining uniformity in polymersome size, thereby limiting commercial viability. Microfluidic techniques may offer a solution to optimize production, though further improvements are needed for industrial-scale reproducibility. To date, the impact of surface modifications (such as ligand conjugation or charge alterations) on the targeting efficiency and cellular uptake of polymersomes in the gastrointestinal tract has yet to be fully elucidated. Mucosal interaction is another concern, as unwanted mucoadhesion could hinder drug delivery to the small intestine. Finally, potential immunogenicity or long-term safety implications from repeated oral administration of polymersomes need thorough investigation. These challenges must be addressed before the widespread clinical application of polymersomes in oral drug delivery can be achieved.

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