MINIREVIEW

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Twenty-five years of polymersomes: lost in translation?

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Soon after the discovery of polymeric vesicles (polymersomes), reports of their high membrane stability raised hopes for the development of next generation vesicles for drug delivery and diagnostic applications. Twenty-five years later, however, liposomes remain the only clinically tested colloidal vesicular formulations. To highlight the translational challenges faced by polymersomes, we critically reviewed a selection of polymersome formulations with a focus on their pharmacokinetic and pharmacodynamic aspects.

1.1 Polymersome definition

Polymersomes (polymersomes, polymeric vesicles, Fig. 1A) are defined as vesicular macromolecular assemblies whose bilayer membrane is composed of amphiphilic polymers (block, dendronized, graft, or alkylated copolymers).[1–4] The hydrophilic corona of the membrane faces the aqueous core and outer aqueous phase. The hydrophobic layer of the membrane separates the inner from the outer medium. The molecular composition and length of the hydrophobic and hydrophilic blocks determine different

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polymersome properties such as membrane rigidity, size, and stability.[5,6] Polymersomes have been the subject of several recent review articles.[7–9]

1.2 Historical perspective

In 1995, two seminal papers in *Science* advanced our understanding of polymeric macromolecular assemblies.[1,2] In the first published study, van Hest *et al.* described different macromolecular structures formed by amphiphilic polymers composed of a poly(styrene) (PS) block and a poly(propylene imine) dendrimer (dendr) in aqueous solution. While PS-*dendr*-$(NH_2)_8$ formed vesicles with diameters below 100 nm (Fig. 1B), PS-*dendr*-$(NH_2)_{16}$ formed micellar rods ($\sim$12 nm in diameter) and PS-*dendr*-$(NH_2)_{32}$ spherical micelles ($\sim$10–20 nm).[2] In the subsequent *Science* issue, an article

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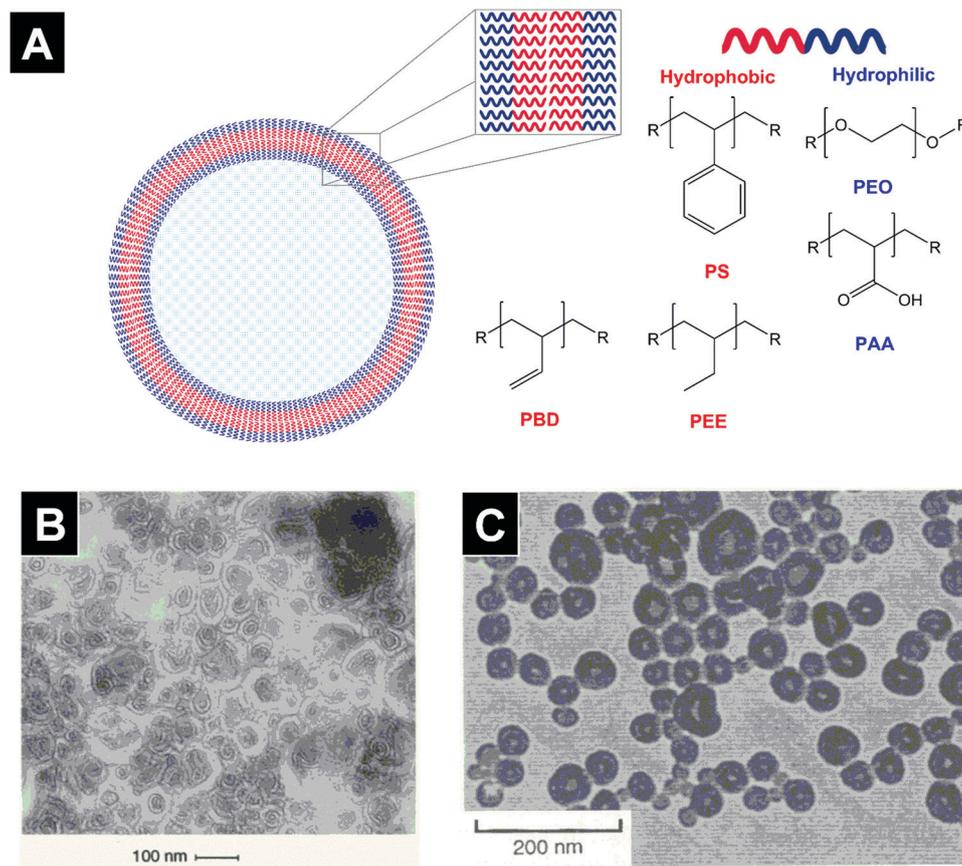
Jean-Christophe Leroux

Fig. 1 Schematic depiction and first reported TEM images of polymeric vesicles in aqueous media. Polymersomes are vesicular structures made of amphiphilic co-polymers (A). In 1995, van Hest *et al.* demonstrated the vesicular morphology of macromolecular assemblies made of PS-*dendr*-(NH₂)₈ on TEM images in aqueous solution (B). Soon afterwards, Zhang and Eisenberg showed that PS-*b*-PAA diblock copolymers formed vesicular structures in a water–DMF mixture using TEM imaging (C). Figures adapted from ref. 1 and 2 with permission. PS: poly(styrene); PBD: poly(butadiene), PEE: poly(ethylethylene), PEO: poly(ethylene oxide), PAA: poly(acrylic acid).

by Zhang and Eisenberg reported the morphological diversity of macromolecular assemblies of PS-*b*-poly(acrylic acid) (PS-*b*-PAA) diblock copolymers in a *N,N*-dimethylformamide–water mixture. Upon decreasing the PAA block length, transmission electron microscopy (TEM) images revealed a transition from spherical (PS$_{200}$-*b*-PAA$_{21}$, 26 nm in diameter) to rod-like micelles (PS$_{200}$-*b*-PAA$_{15}$, 23 nm), to vesicles (PS$_{200}$-*b*-PAA$_8$, 100 nm, Fig. 1C), and large spherical aggregates (PS$_{200}$-*b*-PAA$_4$, up to 1.2 μm). These studies revealed that amphiphilic copolymers were able to form vesicular structures in low molecular weight solvents, and that tuning the hydrophilic to hydrophobic ratio of block copolymers allowed the formation of different macromolecular assemblies. A subsequent study by Zhang and Eisenberg revealed that the macromolecular morphology of a diblock copolymer can be altered by modifying the ionic strength of the medium.[10]

The notion that polymersome membranes are much tougher than liposomal ones was first put forward by Discher *et al.* who showed that poly(ethyl ethylene) (PEE)$_{37}$-*b*-poly(ethylene oxide)$_{40}$ (PEO, also referred to as poly(ethylene glycol), PEG) polymersomes resisted higher areal strains than liposomes made of the unsaturated phospholipid 1-stearoyl-2-oleoyl-*sn-glycero*-3-phosphatidylcholine (SOPC, phase transition temperature $T_m \sim 6$ °C) in micropipette

aspiration experiments.[11] A subsequent study by the same group demonstrated that poly(butadiene)$_{46}$ (PBD)-*b*-PEO$_{26}$, PBD$_{55}$-*b*-PEO$_{50}$, PBD$_{125}$-*b*-PEO$_{80}$, and PBD$_{250}$-*b*-PEO$_{150}$ polymersomes ruptured at an areal expansion of ∼10–40%, while SOPC liposomes ruptured at ∼4%.[5] This study is the origin of the widely propagated notion that polymersomes are considerably more stable than liposomes.[8,12–16] While the evidence put forward by Discher *et al.* clearly showed superior mechanical properties of the investigated polymersomes over the liposomal control, comparisons with additional control formulations need to be performed as clinically used liposome formulations differ greatly from SOPC vesicles both in terms of phospholipid phase transition temperature and cholesterol content.[17] In liposomes, hydrogenation of the phospholipid improves membrane packing and thus leads to increased toughness and lower permeability.[18] Evaluating stability differences among polymersome systems with different glass transition temperatures and biodegradability profiles may further nuance this statement. PBD$_{250}$-*b*-PEO$_{150}$ polymersomes (membrane thickness ∼21 nm) ruptured at lower areal strength than PBD$_{125}$-*b*-PEO$_{80}$ polymersomes (∼15 nm), indicating that neither hydrophobic block length nor membrane thickness are the only determining factors of membrane strength.[5]

Interestingly, poly(ethyl ethylene) (PEE)-*b*-PEO polymersomes showed similar resistance to areal strain (rupture at ∼10%) as PBD-*b*-PEO of a similar molecular weight.[5] Furthermore, investigations of the elastic moduli of various polymersome and liposome formulations demonstrated the strong influence of block copolymer/phospholipid composition and did not show a general superiority of polymersomes.[5,19–27] Finally, the question, which clinical applications necessitate tougher membranes than liposomal ones, also needs to be addressed.

1.3 Preparation methods

Polymersomes can be prepared by various methods. In general, the polymer is dissolved in an organic solvent to form an organic phase that is added to an aqueous solution (aqueous phase). The mixing allows a fine dispersion of the polymer in the water phase and the subsequent formation of polymersomes. Different techniques may be employed for the mixing step: nanoprecipitation, emulsification, or film rehydration. In order to compare these methods quantitatively, the polymersome yield or at least the polymer concentration of the dispersion would be needed. Unfortunately, this information is generally lacking. Furthermore, preclinical and clinical studies necessitate scaled up preparation procedures with high reproducibility and acceptable amounts of residual organic solvents and degradation products. For liposomes, retaining the same physicochemical properties and toxicity profile after scale up remains challenging despite the multitude of FDA-approved formulations.[28–30] The ample industrial experience on tackling the challenges of large-scale liposome production will be highly valuable for polymersome systems, especially if similar procedures (*e.g.*, film rehydration, ethanol injection, extrusion) can be applied.

1.3.1 Nanoprecipitation

Nanoprecipitation is a widely used method for polymersome production.[6,31,32] The polymers are dissolved in a suitable water-miscible organic solvent, to which the aqueous phase is slowly added under stirring, usually with a syringe pump. This method is associated with engineering challenges regarding loading efficiency, scalability, and reproducibility.[33] To address some of these challenges, flash nanoprecipitation, a method used to prepare solid-core nanoparticles from block copolymers, was recently adapted to polymersome preparation.[33] In this method, multi-stream mixers are employed to mix an amphiphilic block copolymer-containing water-miscible organic solvent with an aqueous solution under turbulent conditions, and to subsequently introduce this mixture into an aqueous reservoir.[33] This method enabled the preparation of poly(propylene sulfide)-*b*-PEO polymersomes loaded with hydrophilic (green fluorescent protein, alkaline phosphatase) and hydrophobic cargoes (rapamycin), and promises to yield a scalable polymersome preparation platform once validated for a range of diblock copolymers and larger batch volumes. Unfortunately, flash nanoprecipitation has not yet been shown to solve another issue of nanoprecipitation-based methods, the necessity of diluted conditions (generally below 1 wt%)[6,31–33]

and thus additional concentration steps to prepare concentrated polymersome dispersions.

The complete removal of the water-miscible organic solvents used in nanoprecipitation is of high importance in order to avoid toxicity and vesicular instability, as organic solvents have been reported to act as a plasticizer in the polymersome membrane.[34] Thorough purification procedures such as dialysis or cross-flow filtration coupled with a quantification of residual solvents would be needed.

1.3.2 Emulsification

In the oil-in-water single emulsion method, the polymer-containing, at least partly water-immiscible organic solvent is mixed with the aqueous phase under sonication, homogenization, or vigorous stirring.[35,36] The solvent is then removed by evaporation or filtration methods.[35–37] This method was shown to allow the preparation of PS-*b*-PEO polymersomes with high concentrations (up to 10% w/v) and low solvent residues, making it suitable for *in vivo* experiments.[37] In addition, emulsification-based methods can relatively easily be scaled up and may be performed in a continuous process.[38,39]

In the double-emulsion method, polymersomes form in a water-oil-in-water double emulsion containing an aqueous inner phase, a polymer-containing, at least partly water-immiscible organic solvent in the middle phase, and an aqueous outer phase.[40–42] This method can be elegantly carried out with a microfluidic system with which narrow size distributions in the low micrometer size range can be achieved.[40–42] Microfluidics-based systems for mass production of vesicles were proposed but have yet to prove their capacity to provide large volumes of highly concentrated vesicular dispersions at industrial scale.[43,44]

1.3.3 Film rehydration

In the film rehydration method, the polymer is dissolved in an organic solvent and subsequently dried, allowing the formation of a thin polymer film.[45–47] The polymers self-assemble into vesicles upon addition of the aqueous phase and thorough mixing. This method is widely used in the preparation of liposomes as it yields highly concentrated dispersions with high yields and low residual solvent amounts.[48] The latter are generally not reported for polymersomes prepared with this method,[45–47] and its applicability to vesicles with highly hydrophobic blocks is limited.[49]

1.3.4 Cross-linking

Cross-linking the hydrophobic copolymer fragments generally aims at increasing the stability of the polymersome membrane. Cross-linking procedures have been reported for many polymersome systems such as PBD-*b*-PEO or PEO-*b*-PAA-*b*-poly(*N*-isopropylacrylamide).[50–52] However, the removal of toxic residues from the cross-linking procedure could be challenging as they may be retained in the membrane. If the drug is already loaded, performing a cross-linking procedure could potentially chemically modify the drug.

1.3.5 Size control

The vesicle size affects the uptake by the mononuclear phago-cytic system (which influences circulation time), extravasation,

and organ distribution.[53] Upon oral application, it may further impact the systemic availability due to size-dependent differences in diffusion across the mucin layer and M-cell uptake.[54] Membrane extrusion, sonication, and size-exclusion chromatography can be used to control the polymersome size.[34,55–60] Similarly to liposomes, the size of polymersomes composed of polymers with low glass transition temperature hydrophobic fragments (e.g., poly(propylene oxide), PBD) can readily be decreased applying freeze–thaw and/or extrusion cycles, yielding polymersomes of less than 100 nm in diameter.[55–59] However, for polymers with a high glass transition such as PS-b-PEO, decreasing the size of polymersomes with filtration and sonication has only been reported in the presence of considerable volume fractions of organic solvent.[34] The solvent probably acts as a plasticizer of the semi-crystalline membrane, facilitating macromolecular rearrangements during filtration or sonication. Size-exclusion chromatography was further used to separate fractions of PBD-b-PEO polymersomes of different sizes.[60] For more information on controlling polymersome size, the reader is referred to a recent review on this subject.[8]

1.4 Drug loading

As their lipidic counterparts, polymersomes offer the possibility to host hydrophilic and hydrophobic cargo in the aqueous core and hydrophobic part of the membrane, respectively.

1.4.1 Hydrophilic drugs and membrane proteins

Hydrophilic substrates can be passively or actively loaded into vesicles. In passive loading strategies, the cargo is added to the aqueous phase in which the vesicles form (e.g., film rehydration) or to the inner water phase (e.g., microfluidics). If the substrate is ionizable and efficiently diffuses across the membrane (i.e., low molecular weight and moderately polar weak acids or bases), an active loading method based on a transmembrane pH gradient can be employed. In this approach, the uncharged species of the drug diffuses across the membrane into the vesicular core. The substrate becomes charged and subsequently trapped if the core pH is low (basic cargo) or high (acidic cargo).[61] Very high encapsulation efficiencies and a stable retention can be achieved with this method.[17,62,63]

Leaky membranes impair the stability of the transmembrane pH-gradient and may result in low loading efficiencies and drug leakage after loading. The thicker membranes of polymersomes could therefore be an advantage over the liposomal ones for this remote loading procedure if they remain permeable enough for a sufficiently rapid diffusion of the substrate.

Doxorubicin encapsulation efficiencies of ∼25% and ∼60% were reported for poly(2,4,6-trimethoxybenzylidenepentaerythritol carbonate) (PTMBPEC)-b-PEO polymersomes[64] and polymersomes made of a blend of PBD-b-PEO (75 wt%) and poly(L-lactic acid)-b-PEO (25 wt%).[65] In comparison, liposomes made of saturated phospholipids can reach encapsulation efficiencies of up to almost 100%.[66,67] The drug loading in terms of doxorubicin weight per weight of polymer or lipid was ∼8 wt% for PTMBPEC-b-PEO polymersomes,[64] ∼5 wt% for polymersomes made of PBD-b-PEO blended with poly(L-lactic acid)-b-PEO,[65] ∼5 wt% for PEGylated liposomes made of the saturated phospholipid 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC),[68] and ∼13 wt% for the Food and Drug Administration (FDA)-approved liposomal doxorubicin formulation Doxil© (Table 1).[69,70]

The investigation of doxorubicin leakage during storage at 4 °C revealed strong differences between polymersomes made of a PBD-b-PEO and poly(L-lactic acid)-b-PEO blend and the liposomal Doxil® formulation. While these polymersomes showed a release of ∼5% doxorubicin after one month at 4 °C,[65] the amount of free doxorubicin in Doxil® did not change over six months.[17] With a leakage rate of 5% per month, only ∼36% of the initially encapsulated drug would remain in the core of the polymersomes after twenty months in the fridge (the shelf-life of Doxil®). To enhance the retention of the encapsulated cargo, polymersomes containing highly hydrophobic blocks such as PS may be a better (albeit non-biodegradable) option. PS-b-PEO polymersomes showed a leakage rate of only 1.5% per month of rhodamine B in surfactant-containing solutions[75] and retained a transmembrane pH gradient over five months at 4 °C.[37]

Over thirty years of experience with the transmembrane pH-gradient loading method in liposomes yielded a range of investigative and FDA-approved liposome formulations with high encapsulation efficiency, drug loading, and very low drug leakage rates.[66,76] Therefore, optimized liposomal formulations are highly suitable for the transmembrane pH gradient-based active loading method and able to retain drugs in the core for prolonged periods of time. Clear advantages of the polymersome formulations reported to date are not obvious for this procedure in terms of encapsulation efficiency, drug loading, and drug leakage.

Conjugating small and large molecules to the polymersome-forming block copolymer can be used to encapsulate drugs and decorate polymersomes with targeting ligands.[77–79] Incorporating drugs using a biodegradable linker (analogously to drug–lipid conjugation for liposomes[80,81]) may provide prolonged release kinetics due to an improved retention in the vesicle.[82]

To increase the permeability of polymersomes for hydrophilic molecules and ions in a selective manner, transmembrane

Table 1 Selected FDA-approved intravenous liposome formulations which could serve as controls for investigative polymersome formulations

Trade name	Active pharmaceutical ingredient	Liposome composition (mol%)	First FDA-approved indication (year of approval)
Ambisome®[71–73]	Amphotericin B	Hydrogenated soy phosphatidylcholine (HSPC), cholesterol, distearoyl phosphatidylglycerol (53 : 26 : 21)	Fungal infections and visceral leishmaniasis (1997)
Doxil®[69,70,72]	Doxorubicin	HSPC, cholesterol, mPEO(2000)-distearoyl-sn-glycero-3-phosphoethanolamine (55 : 40 : 5)	AIDS-related Kaposi's sarcoma (1995)
Marqibo®[74]	Vincristine	Sphingomyelin (SM) and cholesterol (55 : 45)	Acute lymphoblastic leukemia (2012)

channel proteins can be incorporated into the membrane. In enzymatic cascade reactions, for instance, enzymes that are encapsulated in channel protein-containing membranes can be spatially separated from inhibiting factors while preserving their access to the substrate, as shown for cytidine-monophosphate-*N*-acetylneuraminic acid synthesis with PMOXA-*b*-PDMS-*b*-PMOXA polymersomes containing the channel protein OmpF G119D.[83] Moreover, the incorporation of an *Escherichia coli* glycerol transporter protein into PMOXA-*b*-PDMS-*b*-PMOXA polymersomes led to an enhanced diffusion of the sugar alcohol ribitol and its enzymatic biosensing in the polymersome core.[84] Transmembrane protein incorporation in polymersomes is further used to provide cell-mimicking systems with different membrane environments than those encountered in liposomes as recently reviewed.[85] In more fundamental membrane protein studies, the chemical versatility of polymersome membranes was used to tune their mechanical properties, allowing for an improvement of membrane protein insertion.[86] As most of these channel proteins have evolved for lipid membranes, however, the thickness of the membrane may theoretically limit the insertion of transmembrane proteins. Interestingly, polymersome membranes seem to adapt their conformation to allow the incorporation of biopores smaller than the hydrophobic membrane layer as shown for PMOXA-*b*-PDMS-*b*-PMOXA systems.[87]

1.4.2 Hydrophobic drugs

A major difference between lipid and polymeric vesicles is the membrane thickness which is limited to ∼3–5 nm for liposomes, while polymersomes can have membranes of up to ∼50 nm in thickness when long block copolymers are used.[75,80,88] Therefore, polymersomes could in theory accommodate larger and higher amounts of hydrophobic molecules than liposomes. In addition, thicker membranes may result in slower release rates of hydrophobic substrates due to higher diffusional distances (Fig. 2). The hydrophobic cargo is generally added to the organic phase and incorporated into the membrane upon vesicle formation (*e.g.*, nanoprecipitation, film rehydration, microfluidics).[88–90]

Using nanoprecipitation at pH 10.5, doxorubicin was loaded into poly(trimethylene carbonate)-*b*-poly(L-glutamic acid) polymersomes with an encapsulation efficiency of 78% and a drug loading of 47 wt%.[90] Transmission electron microscopy indicated that the high drug loading was related to the formation of doxorubicin nanoparticles at the alkaline pH (pK_a 8.3), which were incorporated in the hydrophobic layer of the membrane.[90] Hydrophobically modified iron oxide nanoparticles with a radius of ∼3–5 nm were also incorporated into the membrane of poly(trimethylene carbonate)-*b*-poly(L-glutamic acid) and PEO-*b*-poly(D,L-lactic acid) polymersomes.[31,91] Interestingly, ∼5 nm-thick liposomal membranes made of the saturated phospholipid 1,2-dipalmitoyl-*sn-glycero*-3-phosphocholine (DPPC) can also accommodate oleic acid-derivatized hydrophobic iron oxide nanoparticles of 5 nm in radius.[92] Therefore, the membrane thickness does not seem to be the only determining factor in the loading of large hydrophobic substrates such that liposomal controls could be relevant even in the case of large hydrophobic substrates.

When loading substrates into the membrane, the risk of low drug retention due to the low diffusion distance in the membrane needs to be considered. Even though polymersome membranes are generally thicker than liposomal ones, the diffusion distance remains small, resulting in a risk of rapid cargo release. Indeed, paclitaxel-loaded hydrophobic polymeric nanoparticles with a diameter of ∼180 nm showed a fast release of their hydrophobic cargo due to diffusion.[93] To determine the degree of retention in the membrane, thorough studies on the release kinetics of membrane-loaded substrates are needed in biorelevant media and under sink conditions or, preferably, *in vivo*. Moreover, the use of biodegradable polymers (*e.g.*, for systemic administration) could further impact the release profile *in vivo*, as the degradation of the polymer could destabilize the membrane and result in accelerated cargo release.

Potential advantages

Possible issues

Chemical versatility
Opportunities for controlled release

Thick and highly hydrophobic membranes
Higher loading and better retention of drugs, transmembrane proteins, and nanoparticles

High degree of surface PEGylation
Improved stealth effect

Reduced immunogenicity?

Novel chemical entities
Risk of toxicity of polymer and/or metabolites

Highly hydrophobic copolymer
Polymer aggregation
Hydration can be challenging
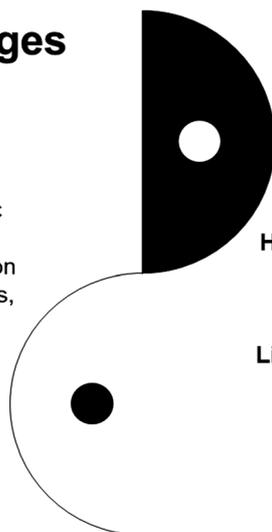
Limited experience for scale-up

Fig. 2 Potential advantages and possible disadvantages of polymersomes *vs.* liposomes.

1.5 *In vivo* studies

1.5.1 Systemic administration

As the membrane strength and surface PEGylation of liposomal systems influence their circulation half-life and leakiness,[66] the initial reports on the high membrane stability of polymersomes raised hopes for longer circulation times and a greater drug retention *in vivo* than their lipidic counterparts.

For liposomes, increasing the membrane stability with cholesterol and high phase transition temperature phospholipids (*e.g.*, DSPC, HSPC) allowed to prolong the circulation half-life and improve the retention of luminal cargo.[94,95] Therefore, in the case of polymersomes, high glass transition temperature polymers may be the preferable hydrophobic polymeric segments when long circulation times and high drug retention are sought. The phase and glass transition temperature of commonly used phospholipids and hydrophobic block are listed in Table 2.

In PEGylated liposomes, the binding of opsonins to the liposomal surface was reduced, the uptake by opsonin-recognizing cells of the mononuclear phagocyte system lowered, and the circulation time prolonged compared with non-PEGylated liposomes.[102] Due to their higher surface PEO density (100 mol% *vs.* generally 0.1–5 mol% for PEGylated liposomes[48,63,103,104]), PEO-containing polymersomes might exhibit a longer circulation half-life than liposomes. This hypothesis is debatable, however, because a PEO content of more than 5 mol% did not improve liposomal surface PEO coverage, blood protein adsorption, and circulation longevity with PEGylated liposomes.[103,105]

PEGylated liposomes elicit an immune response with anti-PEO immunoglobulin M antibodies. These antibodies led to an accelerated clearance upon repeated injection of PEGylated liposomes.[69,106,107] Similar immune responses with an impact on the pharmacokinetic profile were reported for PEGylated polymeric nanoparticles after multiple applications.[107] Interestingly, in a recent study investigating intravenous weekly administrations of poly(propylene sulfide)-*b*-PEO polymersomes in non-human primates, increases in anti-PEO immunoglobulin M or G antibodies were not observed.[108] More studies on immune responses against repeatedly administered PEGylated polymersomes are warranted to confirm and mechanistically understand these

findings. The immunogenicity of other hydrophilic fragments (*e.g.*, poly(oxazoline), PAA) is less well understood.[109]

The number of pharmacokinetic studies on polymersomes is limited. One of the most cited ones investigated the circulation time of four non-biodegradable polymersomes made of PBD-*b*-PEO or PEE-*b*-PEO.[45] To assess their circulation time *in vivo*, these polymersomes were stained with the hydrophobic fluorescent dye PKH26.[45] This dye is conjugated to a C_{14} and a C_{22} chain and generally used for cell staining as the aliphatic chains insert into the lipid bilayer.[110] An investigation of the dye retention in the membrane was unfortunately not reported in this study. The circulation half-lives of the polymersomes ($\sim$120 nm) upon intravenous injection in rats ranged between 16 and 28 h, with longer circulation times observed for the longer diblock copolymers PBD_{54}-*b*-PEO_{50} ($\sim$28 h) and PBD_{130}-*b*-PEO_{80} ($\sim$26 h) than for PBD_{46}-*b*-PEO_{26} ($\sim$16 h).[45] Interestingly, PEE_{37}-*b*-PEO_{40} ($\sim$19 h) polymersomes did not show a longer half-life than their PBD counterparts, even though hydrogenation theoretically leads to a higher membrane stability due to better membrane packing. In another pharmacokinetic study, [111]Indium-labeled PBD_{70}-*b*-PEO_{22} polymersomes of 90 nm showed a circulation half-life of $\sim$20 h in mice as determined with single-photon emission computed tomography.[59] Unfortunately, a direct comparison to PEGylated liposomes was not performed in these studies.[45,59] In the literature, PEGylated liposomes of similar size showed half-lives of 15 to 35 h in rodents.[106,111–115] Therefore, clear evidence of a longer circulation time of polymersomes compared with PEGylated liposomes is currently missing. The impact of the high surface PEO density of polymersomes on half-life remains questionable. Furthermore, further studies on the impact of immunological reactions on the half-life of repeatedly administered polymersomes are warranted.

To address the non-biodegradability of PBD-*b*-PEO vesicles, polymersomes made of a blend of PBD_{125}-*b*-PEO_{80} (75 wt%) and poly(L-lactic acid)$_{56}$-*b*-PEO_{109} (25 wt%) were loaded with doxorubicin in the core and paclitaxel in the membrane (3 mg kg^{-1} doxorubicin, 7.5 mg kg^{-1} paclitaxel) and assessed for their antitumor efficacy. This formulation led to a prolonged tumor doxorubicin exposure and accelerated tumor regression in tumor-bearing nude mice compared with the free drugs (1.5 mg kg^{-1} doxorubicin, 1.0 mg kg^{-1} paclitaxel).[116] Doxorubicin tumors levels

Table 2 Glass (T_g) or phase transition temperatures (T_m) of commonly used hydrophobic co-polymer blocks and lipids

Polymer	T_g (°C)
Poly(butadiene) (PBD)	-92[96]
Poly(ethylethylene) (PEE)	-45[97]
Poly(D,L-lactic-*co*-glycolic acid) (1:1, PLGA)	43[98]
Poly(D,L-lactic acid) (PLA)	52[98]
Poly(styrene) (PS)	97[99]

Lipid	T_m (°C)
1,2-Dioleoyl-*sn-glycero*-3-phosphocholine (DOPC, $[18:1]_2{}^a$)	-17[100]
1,2-Dipalmitoyl-*sn-glycero*-3-PC (DPPC, $[16:0]_2{}^a$)	41[100]
1,2-Distearoyl-*sn-glycero*-3-phosphocholine (DSPC, $[18:0]_2{}^a$)	55[100]
Hydrogenated soy phosphocholine (HSPC, mainly 18:0,^{a} $\sim$11% 16:0^a)	$\sim$49[100]
Sphingomyelin (SM, $[18:1/16:0]^a$)	41[b 101]
1-Stearoyl-2-oleoyl-*sn-glycero*-3-phosphocholine (SOPC, $[18:0/18:1]^a$)	6[100]

^{a} Composition of lipid tails (number of carbon atoms:number double bonds). ^{b} T_m for EggSM from Avanti®.
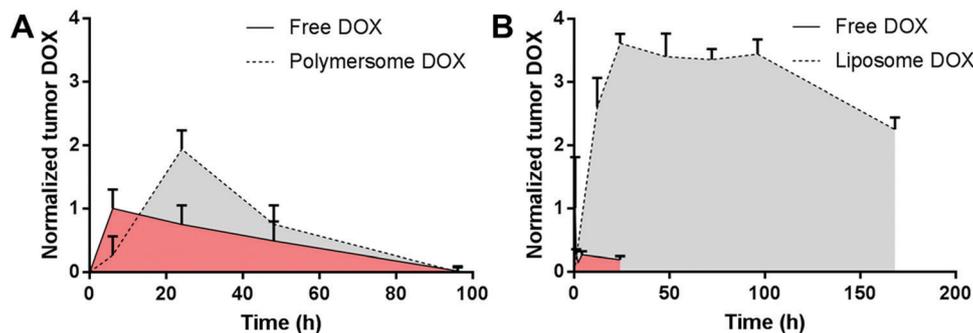
Fig. 3 Comparison of the normalized tumor doxorubicin deposition in mice receiving polymersome or liposome formulations. Polymersome encapsulation led to higher doxorubicin levels compared with the free drug at 24 h (A), while liposomal doxorubicin (Doxil$^{©}$) led to prolonged tumor exposure to doxorubicin for at least seven days (B). Plot A: free and polymersome-encapsulated doxorubicin administered in nude mice bearing MDA-MB231 human Caucasian breast adenocarcinoma tumors of 0.5 cm^2, dose not mentioned, n = 2 per time point.[116] Plot B: free doxorubicin and Doxil$^{©}$ at 9 mg kg^{-1} administered ten days after 4T1 mouse mammary carcinoma tumor inoculation in female BALB/c mice (tumor size not indicated), n = 3–5 per time point.[118] Data normalized in both plots to the highest mean tumor doxorubicin level in the respective free doxorubicin group. DOX, doxorubicin.

of the polymersome formulation peaked after one day and decreased to ~40% and ~0% after two and four days, respectively (Fig. 3A).[116] Unfortunately, paclitaxel levels were neither assessed in the tumor nor in plasma and liposomal controls were not included in this study. The clinically used liposomal doxorubicin formulation Doxil$^®$ could serve as an appropriate positive control due to its high stability (saturated phospholipids, high cholesterol content) and stealth properties (PEGylation).[17,70,117] In a study on Doxil$^®$ in tumor-bearing mice, the liposomal formulation resulted in a much greater and prolonged exposure of the tumor to doxorubicin compared with the free drug (Fig. 3B).[118] The maximum concentration was achieved after two days, remained stable until day five, and was decreased to ~70% on day seven after injection.[118] This lower and shorter tumor doxorubicin exposure in the case of the polymersomes could be due to a shorter plasma half-life of the polymersomes or a higher leakage of doxorubicin before reaching the tumor. Unfortunately, the plasma half-life of the PBD-b-PEO/poly(L-lactic acid)-b-PEO polymersome system was not calculated in the study.[116] Comparing the half-life of similar polymersomes (PBD$_{130}$-b-PEO$_{80}$, ~26 h[45]) with Doxil$^®$ liposomes (~35 h,[112] both in rats), strongly different plasma clearance profiles in mice would not be expected. Furthermore, a comparison of the absolute tumor accumulation of the doxorubicin-loaded vesicles cannot be made because the tumor doxorubicin levels were not quantified in terms of amount of drug per amount of tumor tissue in the polymersome study.[116] This example, in which a liposomal formulation led to a longer exposure of the tumor to the chemotherapeutic agent, underlines the importance of liposomal controls in the evaluation of novel polymersome formulations.

To demonstrate the polymersomes' capacity to host large hydrophobic substrates in their membrane, oligo(porphyrin) near-infrared dyes were incorporated in PBD-b-PEO polymersomes.[88] These highly rigid and hydrophobic dyes were added to the polymer film and encapsulated in the membrane during rehydration.[88] While the polymersomes could incorporate up to pentameric oligo(porphyrin), liposomes made of SOPC could only accommodate dimers in their membrane.[88] The authors speculated

that the trimeric to pentameric oligo(porphyrin) were too large (~3–5 nm) for the liposomal (~3.5 nm) compared to the polymersome membrane (~10 nm).[88] However, other factors could have influenced the loading procedure as the membrane of DPPC liposomes was capable of incorporating hydrophobic nanoparticles of ~5 nm.[92] Upon intratumoral injection into subcutaneous glioma-bearing rats, a strong fluorescence signal of the oligo(porphyrin)-loaded polymersomes was observed in the investigated 20 min post-injection interval.[88] In view of the missing positive and negative controls in the in vivo study, the added value of a polymersome-based delivery system over other platforms cannot be judged.

To target the hyaluronic acid-binding glycoprotein CD44 on cancer cells, a study used hyaluronic acid as the hydrophilic copolymer fragment. Doxorubicin-containing poly(γ-benzyl-L-glutamate)-b-hyaluronic acid polymersomes were taken up by CD44-expressing breast cancer cells in vitro.[119] These polymersomes decreased the tumor size of breast cancer-bearing rats in vivo more than free doxorubicin[119] but a liposomal doxorubicin control formulation (e.g., bearing surface-exposed hyaluronic acid[120]) was missing. The encapsulation of doxorubicin into polymersomes further decreased its cardiotoxicity[119] which was already reported for liposomal doxorubicin in 1984.[121]

Cytosol-targeting polymersomes were developed for the delivery of cyclic dinucleotide agonists of the immunomodulatory protein stimulator of interferon genes for cancer immunotherapy.[122] These chemotherapeutic drugs exhibit an unfavorable pharmacokinetic profile due to rapid systemic clearance and low cell permeability.[122] The polymersomes were made of PEO-b-poly(2-(diethylamino)ethyl methacrylate-co-butyl methacrylate-co-pyridyl disulfide ethyl methacrylate) (PEO-b-poly[DEAEMA-co-BMA-co-PDSMA]) copolymers.[122] The PDSMA block allowed for an improved cargo retention after crosslinking via disulfide bridge formation while providing a redox-responsive element which is destabilized in the supposedly increasingly reducing environment of the endosome.[122] However, the extent of endosomal bio-reduction is disputed.[123–125] Another destabilizing moiety in the hydrophobic part of the membrane are the DEAEMA

units whose amines get protonated upon acidification in the endosome.[122] In combination with the hydrophobic BMA block, the positive charges aimed at destabilizing the endosomal membrane to promote endosomal escape.[122] Intratumoral injection of these polymersomes into B16–F10 melanoma-bearing mice decreased the tumor growth and induced remission in approx. one third of the animals over the 60 day study.[122] Systemic administration led to lower antitumor effects, and remission was only achieved in combination with immune checkpoint blockade.[122] This example provided an interesting attempt to use the versatility of the hydrophobic polymer fragment to increase drug retention while promoting polymersome destabilization in the endosome and endosomal escape. A liposomal system with these membrane properties is difficult to imagine due to the lower versatility of phospholipids.

Another polymersome system with a disulfide-bond cross-linked membrane was recently proposed for the treatment of ulcerative colitis.[126] The polymersomes were composed of PEO-b-poly(trimethylene carbonate-co-dithiolane trimethylene carbonate)-b-poly(ethyleneimine) and PEO-b-poly(trimethylene carbonate-co-dithiolane trimethylene carbonate) modified with a macrophage-targeting peptide.[126] The polymers self-assembled into asymmetric vesicles with of the cationic poly(ethyleneimine) facing the aqueous core, leading to an almost neutral surface charge.[126] The polymersomes were loaded with a silencing ribonucleic acid against tumor necrosis factor-alpha and dexamethasone sodium phosphate.[126] In a biodistribution study, the polymersomes with the macrophage-targeting peptide homed to the colon to a significantly higher extent than the peptide-free vesicle control after intravenous application.[126] In an ulcerative colitis mouse model, the double-loaded polymersomes led to a significant downregulation of tumor necrosis factor-alpha and attenuated several markers of ulcerative colitis disease activity.

Polymersomes with reversible sugar-binding capacity were developed for diabetes therapy.[127] PEO-b-poly[(7-(2-methacryloyloxyethoxy)-4-methylcoumarin)-$stat$-2-(diethylamino)ethyl methacrylate-$stat$-(α-D-glucopyranosyl)ethyl methacrylate] (PEO-b-poly-[CMA-$stat$-DEA-$stat$-GEMA]) glycopolymersomes were loaded with the leptin ConA to enable glucose binding.[127] ConA was retained in the membrane due to its affinity for glucopyranosyl, electro-static interactions with DEA, and the cross-linking of the CMA membrane fragment.[127] Because of the higher affinity of ConA for glucose than glucosyl group, these ConA-loaded glycopolymersomes took up and released glucose depending on the sugar concentration of the outer phase.[127] Intravenous administration of $\sim$5 mg kg^{-1} glycopolymersomes with $\sim$30 mg kg^{-1} ConA in type I diabetic mice led to a decrease in blood glucose to normal levels for approximately 36 h. Unfortunately, the glucose-binding capacity of the formulation was not reported such that theoretical calculations on the administered dose and observed glucose-lowering effects cannot be carried out. In $vivo$ studies on dose finding and dependence were also missing. Further studies are warranted to investigate the system's performance in rapid glucose bursts ($e.g.$, postprandial glucose peaks) and potential immune-mediated effects on circulation time and sugar-binding capacity upon repeated administration.

Iodine-loaded polymersomes were investigated for computed tomography imaging and radioisotope therapy of breast cancer.[128] After intravenous injection to mice, ^{125}I-radiolabeled poly(iodinated carbonate)-b-PEO polymersomes (approx. 100 nm) mainly distributed in organs of the mononuclear phagocytic system and in 4T1 murine breast cancer tumors.[128] Using a two-compartment pharmacokinetic model, a blood circulation half-life of over 10 h in the second phase was calculated.[128] In contrast, free sodium iodate accumulated in the thyroid and was rapidly cleared by the kidneys.[128] A linear signal increase in the tumor was observed over 20 h on computed tomography after intravenous application of ^{125}I-radiolabeled polymersomes.[128] Substituting ^{125}I for the beta emitter ^{131}I led to the development of polymersomes with antitumoral properties.[128] This study illustrates how the chemical versatility of polymersomes can be used to design systems with built-in therapeutic and diagnostic moieties.

In conclusion, convincing evidence that polymersomes circulate longer while retaining higher amounts of cargo than liposomes is still lacking for most systems and applications. With regard to the established clinical safety profile of liposomes, undertaking the challenging process of bringing polymersomes to the clinic seems only worthwhile if two conditions are fulfilled: the identification of applications necessitating polymersome-specific features and a clear demonstration of these advantages for the selected polymersome formulation over relevant liposomal controls. Unfortunately, head-to-head comparisons of polymersomes with well-established liposome formulations (Table 1) with high membrane toughness (high cholesterol content, saturated lipids) and stealth properties (PEGylation) are not reported in most of the reviewed studies.

1.5.2 Oral administration

The gastrointestinal (GI) tract is a harsh environment for vesicles.[129] High bile salts concentrations, strong osmolarity and pH changes, and high enzymatic activity generally lead to rapid destabilization of vesicular structures.[129–133] Bile salts impaired the membrane integrity of liposomes by insertion into the outer leaflet of the membrane and by the partitioning of the lipids into bile salt micelles, which may lead to cargo release.[133–138] Hypo- and hyperosmolar environments, which can range from 100 to 600 mOsmol kg^{-1} in the small intestine,[139–141] induce morphologic changes ($i.e.$, shrinking and swelling, respectively) of vesicles,[142,143] and potentially impair the structural integrity of the membrane. Polyester-based biodegradable polymersomes, surface-exposed peptide-based targeting ligands, and phospholipid-based liposomes may further be hydrolyzed in the acidic environment of the stomach or due to enzymatic action ($e.g.$, pepsin, (chymo)trypsin, phospholipase A$_2$ for phospholipids).[130,144,145] The environment of the colon is also destabilizing for vesicles as the water resorption thickens the chyme and increases the osmolarity.[37] As even PEGylated liposomes with high phase transition temperature phospholipids and cholesterol content do not resist these conditions,[37] oral delivery could be a promising field for highly stable polymersome formulations.

PS-b-PEO polymersomes were investigated by our group as an oral ammonia scavenger to treat hyperammonemia, a serious

complication of liver disease.[37] The weak base ammonia can be efficiently and selectively captured by transmembrane pH-gradient vesicles with an acidic core.[37,48,146,147,161] While neither cholesterol-containing PEGylated DPPC and DSPC liposomes nor PBD-b-PEO polymersomes were resistant to physiologically relevant bile salt concentrations, PS-b-PEO polymersomes preserved their ammonia capture capacity in bile salt-containing media at extreme osmolarity levels and in digestive enzyme-containing fluids.[37] Despite performing well in commonly used GI-simulating fluids, these polymersomes proved to be unstable in dietary fiber-based hydrogels and failed to decrease plasma ammonia levels in hyperammonemic rats. This study underlines differences in stability between liposomes and polymersomes and among polymersomes. It further points to shortcomings of commonly used simulated GI fluids in the evaluation of vesicles for oral delivery,[138,148,149] and highlights the need to account for the colonic environment in vesicle-based oral detoxification.

To demonstrate the solubility enhancement of a poorly soluble small molecular drug with a polymersome formulation, the multikinase inhibitor sorafenib was encapsulated in the hydrophobic part of the membrane of PBD-b-PEO polymersomes and orally administered to healthy mice.[150] The area under the plasma concentration vs. time curve of the polymersome formulation was higher compared to the control formulation (sorafenib suspension).[150] As PBD-b-PEO polymersomes are not stable in surfactant-containing media,[151] differences in luminal bile salt concentrations may impact on the sorafenib release profile. Other drug delivery systems such as pH-sensitive Eudragit® nanoparticles or poly(N-vinylpyrrolidone-vinyl acetate)-containing tablets also showed high oral bioavailability for sorafenib and could serve as alternatives to polymersomes for this drug.[152,153]

A study reported that orally applied insulin-containing dextran-b-poly(D,L-lactide-co-glycolide) polymersomes led to appreciable systemic insulin levels and decreased glucose levels in diabetic mice.[154] In view of the difficulty of reaching the systemic circulation with macromolecular drugs due to the mucus layer and the tight gut epithelium,[54] an in-depth investigation of the translocation from the gut lumen into the systemic compartment is needed to fully understand these results.

1.6 Polymersomes as reaction compartments

As different hydrophobic blocks impact on membrane rigidity and hydrophobicity, the polymersome membrane can be tuned for selective permeability.[56,155,156] Polymersomes have therefore been developed as selectively permeable reaction compartments for diagnostic use and synthetic applications. We illustrate this application with two polymersome systems described above whose use was extended to the diagnostic field. For a detailed discussion of the use of polymersomes as micro- or nanoreactors and their use in diagnostics and theranostics, the reader is kindly referred to recent reviews.[157–160]

The usefulness of transmembrane pH-gradient PS-b-PEO polymersomes to quantify ammonia in solution was recently

demonstrated by our group.[37] The influx of the weakly basic ammonia into the acidic vesicular core led to an increase in core pH which was quantified by an encapsulated pH-sensitive fluorescent dye.[37] The high hydrophobicity of the PS-b-PEO polymersome membrane allowed for a high selectivity to ammonia compared to other weakly basic metabolites and drugs.[37] In contrast, the permeability of cholesterol-containing PEGylated DPPC liposomes towards weakly basic drugs was higher.[147]

The reversibly glucose-sequestering PEO-b-poly(CMA-stat-DEA-stat-GEMA) glycopolymersomes described above were further used as a glucose assay.[127] As they swelled from 400 to 800 nm upon exposition to glucose solutions ("glucose sponge"), the authors proposed a particle size-based glucose-sensing system.[127] This system was neither validated in blood or plasma nor selective to glucose in the presence of mannose.[127] Moreover, the practicality and reliability of particle size measurements compared with well-established point-of-care glucose tests remains questionable.

1.7 Conclusion

Polymeric vesicles were initially described in two seminal publications by van Hest et al. and Zhang and Eisenberg in 1995. Four years later, Discher et al. showed that PEE-b-PEO polymersomes had tougher membranes than low phase-transition temperature liposomes made of SOPC. This study raised high hopes for the development of a new generation of vesicular drug delivery systems that are superior to liposomes in terms of stability, circulation time, and drug loading capacity. Twenty years later, there are still open questions about the general-izability of the higher membrane strength beyond the tested formulations. The very few studies on circulation time pointed to similar half-lives of polymersomes and PEGylated liposomes. A clear superiority in the loading capacity for commonly used hydrophilic or hydrophobic drugs has not been established for polymersomes, and the impact of membrane biodegradability on plasma half-life, drug release, and polymersome stability in general remains poorly investigated. Furthermore, finding upscalable polymersome preparation procedures leading to very high polymersome concentrations and low amounts of residual solvent remains challenging, especially if the experience on the industrial production of liposome cannot be applied (e.g., for highly hydrophobic copolymers). In our opinion, more systematic studies comparing polymersomes of various composition against strong liposome controls are needed to demonstrate the proposed superiority of polymersomes. Only when such advantages are clearly established and relevant clinical applications necessitating these properties are identified, can it be judged if the challenging clinical development of an alternative vesicular drug delivery platform to liposomes, whose clinical safety profile is well established, is warranted.

Abbreviations

DOPC	1,2-Dioleoyl-sn-glycero-3-phosphocholine
DPPC	1,2-Dipalmitoyl-sn-glycero-3-phosphocholine

DSPC	1,2-Distearoyl-*sn-glycero*-3-phosphocholine
FDA	Food and Drug Administration
HSPC	Hydrogenated soy phosphocholine
PAA	Poly(acrylic acid)
PBD	Poly(butadiene)
PEE	Poly(ethylethylene)
PEO	Poly(ethylene oxide)
PLA	Poly(lactic acid)
PLGA	Poly(lactic-*co*-glycolic acid)
PTMBPEC	Poly(2,4,6-trimethoxybenzylidenepentaerythritol carbonate)
PS	Poly(styrene)
SM	Sphingomyelin
SOPC	1-Stearoyl-2-oleoyl-*sn-glycero*-3-phosphocholine
TEM	Transmission electron microscopy

Conflicts of interest

JCL and SM are co-inventors on patents/patent applications related to liposome and polymersome formulations for treating and diagnosing hyperammonemia. These patents have been licensed to Versantis AG. JCL is a shareholder of Versantis AG.

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