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Polymersome-based drug-delivery strategies for cancer therapeutics

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Abstract

Polymersomes are stable vesicles prepared from amphiphilic polymers and are more stable compared with liposomes. Although these nanovesicles have many attractive properties for *in vitro/in vivo* applications, liposome-based drug delivery systems are still prevalent in the market. In order to expedite the translational potential and to provide medically valuable formulations, the polymersomes need to be biocompatible and biodegradable. In this review, recent developments for biocompatible and biodegradable polymersomes, including the design of intelligent, targeted, and stimuli-responsive vesicles are summarized.

The idea of polymeric vesicles (polymersomes) germinated during a discussion about the phase behavior of di-block copolymers in 1994 [1]. This discussion was followed by preparing nanosized polymer vesicles using a specific di-block copolymer (polyethyleneoxide-polyethylene; $f_{EO} = 0.39$, $M_w = 3600$) [2]. These vesicles were proven to be more robust and less water permeable compared with phospholipid vesicles or liposomes [2]. Since then, several research laboratories have been studying polymersomes for different purposes, including drug/gene delivery, diagnosis, bioreactors and cell/viral capsid mimicking [3,4].

These hollow vesicles (Figure 1A) consist of a watery interior that is separated from the aqueous surrounding media by an amphiphilic polymer bilayer. The thickness of the bilayer (5–30 nm) usually causes a more robust and impermeable wall compared with the liposomal structures (3–5 nm) [3]. This feature depends on the molecular weights of the copolymers used in the polymersomes. It has been observed that the membrane thickness (d) is proportional to $MW^{0.55}$.

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Among the biomedical applications for polymersomes, drug/gene delivery holds the most promise due to the tunable chemistry of the block copolymers (including the versatility of monomers and the possibility to change block polymers' molar mass and percentage), their low critical aggregation concentration, and the robustness of the polymersomes' bilayer. The latter characteristic can increase the stability of encapsulated compounds for a long time [5]. The polymersomes' hollow core can be used to encapsulate hydrophilic compounds, and the bilayer can be dedicated for loading the hydrophobic compounds. In such a scenario, the combination therapy (Figure 1B) and diagnostic purposes can be achieved using the polymersomes.

Designing clinically applicable polymersomes has been a challenging area in the last several decades. Herein, we elaborate on the recent developments of biocompatible polymersomes as targeted delivery vehicles for cancer therapy.

Polymersome preparation

Typically, polymersomes are prepared from amphiphilic, linear block copolymers [6]. The ratio of the hydrophilic part to the total mass of the copolymers (f value) is a determinant factor in the formation of different nano structures [6–8]. If the f value is higher than 50%, the formation of micelles is possible, and if the value is 40–50%, worm-like structures are more likely. However, if the f value is between 25 and 40%, which is similar to natural phospholipids, the formation of vesicles is more favorable [6,8] (Figure 2). In addition to linear block copolymers, microarm polymers have been used as alternative building blocks for polymersomes [9,10]. These Y-shaped, complex polymers not only have a greater ability to form polymersomes, but are also able to mimic the phospholipid structures [9,10].

Several methods have been used to prepare polymersomes, including the solvent-exchange method, film rehydration, electroformation [2], and the double-emulsion strategy [11]. Electroformation has been used to construct giant polymersomes. Double emulsion (which have been prepared using capillary microfluidics) is a reliable method for preparing polymersomes with acceptable monodispersity in size and uniformity in the bilayer membrane. Among these methods, the solvent-exchange method is widely used for its ease, reproducibility, and control over the size of nanoparticles. Although filter extrusion has been used to decrease the polydispersity index of liposomes, this method seems to be a very time-consuming and difficult approach for polymersomes, even at elevated temperatures due to the polymersome membrane's robustness.

Drug encapsulation in polymersomes

Drug loading capacity is one of the important factors to be considered in any nanodelivery systems' preparation. If the encapsulation efficiency is not high enough, higher amounts of the nanoparticles need to be injected (to reach the therapeutic window), leading to the introduction of a higher fraction of polymer (in case of polymersomes) in the patient's body. Both passive and active (remote) loading strategies have been utilized for encapsulating hydrophobic or hydrophilic compounds in the polymersomes. For passive loading, the hydrophobic compound of interest is solubilized/dispersed in an organic solvent along with the polymers used for the polymersomes. Hydrophilic drugs or imaging agents are usually

added to the aqueous buffer during polymersome preparation. However, due to the low water solubility of some compounds, and the pivotal need for high drug loading capacity, active liposomal loading procedures (e.g., salt and pH gradient) have been adapted for polymersomes. Preparation of paclitaxel encapsulated polymersomes (from pre-formed vesicles) revealed that drug loading efficiency is about tenfold higher than that of liposomes. The higher efficiency is attributed to the presence of thicker hydrophobic membrane in polymersomes' structure compared with the liposomal lipid bilayer [12]. The hydrophilic, cytotoxic drug, doxorubicin, was encapsulated inside the polymersomes' hollow core using either a pH gradient [12–14] or an ammonium sulfate gradient [15]. The encapsulation efficiency for polymersomes was similar to the corresponding liposomal formulations. Due to the stability and reduced leakage of incorporated compounds, polymersomes have the potential to be more acceptable drug carriers compared with liposomes.

Biomedically translatable polymersomes

Ideal polymersomes with biomedically applicable properties need to have enough loading capacity for the active ingredients. Also, these polymersomes should be robust enough to protect the payload from unexpected release. These nanostructures should have a long circulation time and low renal clearance rate while the cargo release needs to be controllable. In addition, specificity toward the desired target site as well as presenting no or minimum immunogenicity are important criteria. Any premature release of the encapsulated drug can increase the risk of side effects, especially in the case of cytotoxic anticancer drugs. However, reduced therapeutic efficacy and drug resistance could be expected as a consequence of slow drug release. Considering these facts, many studies focus on designing polymersomes with improved biodegradability and biocompatibility.

Biocompatibility

To increase the polymersomes' biocompatibility, interactions with blood components should be adjusted to be at minimum, and the vesicles should be guided toward the target sites. There are several approaches, including PEGylation and using targeting moieties, to improve the pharmacodynamic and pharmacokinetic properties of theranostic delivery systems.

PEGylation

The Enhanced Permeability and Retention (EPR) effect is one of the mechanisms (passive targeting) used for delivering drugs to various cancer tumors. With most tumors, the presence of a nonorganized, leaky vasculature along with gaps of 100 nm–2 μ m have been established. Therefore, nanoparticles with a mean size less than 200 nm are able to extravasate through these leaky, imperfect vasculatures with most cancers. A poorly developed lymphatic system in the tumor tissues helps retain nanoparticles in tumor area. However, it should be noted that blood flow to the tumor site is also restricted in comparison with normal organs. Hence, having a good amount of nanoparticle accumulation requires a long circulation time which, itself, is confined by the body's immune system.

The innate immune system is constantly monitoring the body's environment, searching for foreign materials, including pathogens and other exogenous particles such as drug-delivery systems. Therefore, one of the important challenges with drug-delivery approaches is the fate of the carrier and its ability to escape from the reticuloendothelial system's uptake. If the nanocarrier's surface is not covered to evade the innate immune system, blood proteins (e.g., antibodies and complement system) can surround the carrier (opsonization) and make it engulfable by phagocytes in the reticuloendothelial system's (RES) organs. For example, Kupffer cells (resident liver macrophages) are able to distinguish and process the opsonized foreign particles using scavenger receptors. As a result, the half-life of the drug-delivery systems decreases drastically, and more than 50% of the injected dose is usually cleared shortly after administration [16]. Furthermore, accumulation of the drug-delivery systems by phagocytes in secondary immune organs such as the spleen and liver increases the chance of organ toxicity, decreasing the drug's therapeutic index (Figure 3). Hence, different hydrophilic polymers (e.g., poly(ethyleneglycol) [PEG] and *N*-(2-hydroxypropyl) methacrylamide [HPMA]) [17] and polysaccharides (e.g., heparin) [18] have been used to sterically hinder the opsonization process by the white blood cells. PEG with no charge and a nonbiodegradable [19] characteristic is the most-studied polymer that contributes to the stealth properties of drug-delivery systems.

The PEG surface concentration is also important for imparting stealth properties to the nanocarriers. It has been shown that increasing the PEG surface concentration weakens the protein adsorption on the nanoparticles' surface [20]. Another determinant factor which affects the vectors' camouflage is the size of the PEG polymer. For example, a PEG brush with a molecular weight of more than 3400 Da has the highest effect to decrease human plasma protein adsorption on the surface of polystyrene particles [20]. Electrostatic properties of the vehicle surface area are another determinant factor that should be considered. A slightly positive or negative charge may increase the half-life of drug-delivery systems. However, a highly charged surface should be avoided on the carriers because it not only confers toxicity [21], but also decreases the nanoparticle circulation time by increasing the amount of hepatic uptake [22].

It should be noted that the stealth properties rendered by the hydrophilic polymers are only effective for a limited time. Eventually, proteins adsorb on the nanocarriers' surface, and clearance of polymersomes is unavoidable [6]. In addition, a long circulation time increases the drug's side effects in healthy tissues [16] and may even initiate immune reactions toward the nanoparticles [23]. To further complicate the situation, PEGylation not only decreases the protein adsorption, but also impairs the nanocarriers' interaction with the cells. An interesting solution for this problem is to use sheddable PEG groups [16,24]. With this strategy, PEG helps to achieve a long circulation time; however, the layer sheds after extravasation and increases the chance of the cellular uptake. The other strategy to circumvent the low cellular uptake of PEG-conjugated nanoparticles is to conjugate cell-targeting moieties to the PEG's terminal hydroxyl group.

Targeted drug delivery

Another strategy to alter the biodistribution is active targeting via the incorporation of suitable moieties on the polymersomes' surface. Active targeting can increase the vesicles' selective cellular uptake and decrease the unwanted toxicity to nearby cells/tissues.

Polymersome functionalization has been carried out using proteins (including monoclonal antibodies and antibody fragments), peptides, small organic molecules, carbohydrates and aptamers. Functionalized polymersomes have been used for theranostic purposes with different diseases such as cancers, inflammations and brain-related disorders (Table 1).

Overexpression of various cell surface receptors in malignancies and other disorders (such as inflammations) has been used for designing theranostic delivery systems. For instance, the expression of epidermal growth factor receptor increases on the cell surface for breast, colorectal, and lung cancers [41]. Hence, the anti-HER2 receptor antibody (trastuzumab) has been used to specifically target cancer cells (breast cancer cells metastasized to the bone) and to deliver contrast agents for magnetic resonance imaging that employs polymersomes [25]. Immobilizing different antibodies is an interesting strategy, and several researchers have used this approach to target the proper cells/tissues. In this scenario, the antibody is usually immobilized via a covalent bond on the distal end of PEG block to avoid steric hindrance and to promote specific binding. Using antibodies as targeting agents has some disadvantages. These rather-large proteins are mostly immunogenic, and multifunctionalization of one carrier using antibodies is not favorable because of the antibodies' large size. In addition, developing monoclonal antibodies is fairly expensive and time consuming.

The other widely used targeting agents are peptides. Peptides are smaller and less immunogenic than antibodies, and attaching several peptides on the surface of a single polymersome is possible. Like antibodies, some peptides (e.g., Tet-1 [31] and PR_b [32]) can be used to target a specific cell's surface receptors or molecules. Moreover, some peptides, such as the transactivator of transcription (TAT, derived from the human immunodeficiency virus), and many other chimeric peptides, such as transportan [42], are known as cell-penetrating peptides. Some peptides guide the carriers toward subcellular compartments such as the nucleus and mitochondria. For instance, a heptapeptide derived from the Simian virus SV40 T-antigen has been used to construct nuclear-targeted delivery systems. These rather-new, nuclear-targeted drug-delivery systems are promising approaches that can increase drug efficacy, especially in terms of cancer therapy [43], because they are targeted toward the cell nucleus which contains genetic material. However, there are many barriers that the carrier should overcome to reach the nucleus.

Carbohydrates play important roles with several biological processes, such as fertilization, cell-cell interactions, immune response, and signal transduction [44]. Hence, polymersomes that are conjugated to carbohydrates can be used for the targeted delivery of drugs/imaging agents. For example, sialyl Lewis x (sLex), a carbohydrate which binds to selectins, has been conjugated to polymersomes as a way of targeting the inflammatory tissues [1,37].

Small molecules are sometimes preferred as targeting groups because of their diversity, low molecular weight, small size, low immunogenicity, and inexpensiveness [45]. For example,

folate (Vitamin B₆) is a small, organic molecule that has been widely used for targeted delivery systems because of the folate receptor's overexpression on the cells' surface with cancers of the ovary [46,47], urinary bladder [48], lung [49], breast [50] and colon [51].

Biodegradability

One drawback of polymersomes is the slow content release due to the robust and stable bilayer membrane [14,52]. To control the drug release and increase the drug's bioavailability at the target site, stimuli-responsive polymersomes have been used extensively. These smart nanostructures can receive, transfer, and respond to stimuli effectively. Both external and internal stimuli have been tested for triggering the cargo release. External stimuli can be light [53] or a magnetic field [54], whereas pH [9,55–56], temperature [57], and redox potential [8] are examples of internal stimuli. Using internal stimuli appears to be easier, cheaper, and more applicable [5]. Table 2 illustrates recently studied biodegradable polymersomes and their stimuli.

pH-responsive polymersomes

Due to the presence of a slightly acidic microenvironment in the tumor's extracellular matrix (pH $\approx$ 6.8), inflammatory tissues (pH $\approx$ 6.8), and some subcellular compartments such as endosomes and lysosomes (pH $\approx$ 5–6), pH-sensitive polymersomes have been widely used as smart drug-delivery formulations [55,60]. The acidic pH for the tumor's microenvironment results from the presence of large amounts of lactic acid and ATP hydrolysis in the absence of enough oxygen (hypoxia) as well as the fast metabolism of tumor cells. Because this difference is small, the nanovesicles should be able to quickly respond to small, environmental pH changes.

Forming these stimuli-responsive vesicles can be achieved using different strategies. One approach is to use biodegradable polymers [poly (lactic acid) or poly (ϵ -caprolactone)] as the hydrophobic part of amphiphilic copolymers that are known to undergo acid-induced hydrolysis and the effective release of cargo *in vitro/in vivo* [12]. An alternative approach is to use linkers containing acid-labile groups such as acetal [61] and orthoester [62] in the polymersomes' structure. Finally, introducing proton-buffering components in the formulations has been a successful method for a fast, pH-triggered release of encapsulated materials [10]. With the latter approach, pendant acidic (carboxylic acids/sulfonic acids) or basic groups (amine) undergo protonation or deprotonation following pH alteration. Consequently, the structural transition can induce the formation/deformation of polymeric vesicles, which confers a higher therapeutic index as a result of the fast release of therapeutics at the target site. As an example, the formation of polymersomes containing pH-sensitive, pendant-weak basic groups takes place when the environmental pH is higher than the pK_a for basic groups of the block copolymers (where these groups are uncharged). However, when the pH decreases, the basic groups are protonated, and vesicle integrity is compromised due to the electrostatic repulsions.

A major obstacle that may prevent the application of pH-sensitive polymersomes is the slow response to the stimulus, resulting in a slow drug release, which may eventually induce drug resistance in the adjacent cells. Hence, these nanovesicles not only need to retain the

payload in physiological pH (≈ 7.4) during long circulation times, but also need to respond quickly as a result of the decreased pH at pathological locations.

Redox-responsive polymersomes

Redox potentials are different for the intra/extracellular environments, and this difference tends to be augmented with the solid tumors of ovary, breast, lung, and head-and-neck cancers [63]. The redox potential is controlled by the concentration of glutathione (GSH, L- γ -glutamyl-L-cysteinylglycine, a tripeptide), free cysteine and free homocysteine. Although free cysteine and homocysteine have roles in redox equilibria, GSH has been used more frequently as a stimulus for triggered content releases from polymersomes. GSH is the most prevalent reduced thiol source, and there is a large inherent heterogeneity for intracellular (0.5–10 mM) and extracellular (10–40 μ M) GSH concentrations. Hence, extracellular fluid is, naturally, an oxidizing environment. Both the extracellular oxidative environments [64] and intracellular reductive environments [65] have been utilized for polymersomes' drug release.

Different strategies have been used to construct redox-sensitive drug carriers. Bioreducible polymersomes that possess disulfide bonds respond to the redox potential changes by reversible bond breakage. The number and location of the incorporated disulfide bonds allow fine-tuning of the release property from the vesicles. A typical composition that has been used widely for preparing redox-sensitive micelles [66] and liposomes [67] has been successfully implemented with polymersomes [8]. These copolymers contain a disulfide bond to connect the hydrophobic and hydrophilic polymers. Reductive conditions destabilize the formulation and trigger the release of the encapsulated payload. Cross-linked polymersomes display increased stability and, at the same time, are responsive to a reducing environment [68]. Although the disulfide bond in the polymersomes' backbone increases the drug release in the reducing environments, premature payload leakage may occur due to prolonged circulation with low concentrations of reducing agents in the blood that may decrease the therapeutic efficacy of the redox-sensitive polymersomes.

Temperature-responsive polymersomes

Temperature-sensitive polymersomes are composed of thermosensitive polymers which undergo phase transition with environmental temperature changes. The phase transition causes changes in hydrophilicity/hydrophobicity and alters the polymers' solubility, resulting in vesicle formation/disruption. Thermosensitive polymers have two different groups: polymers with an upper critical solution temperature (UCST) that become soluble with an increased temperature and polymers with a lower critical solution temperature (LCST) which become insoluble as a result of increased temperature. LCST is also called the clouding point, and a phase transition takes place at this temperature [69]. These polymersomes are usually prepared by changing the temperature of a water-solubilized polymer. One advantage of this strategy is the absence of organic solvents in the preparation process for these polymersomes and the lower possible toxicity compared with the solvent-exchange preparation procedure. For instance, with block copolymers containing poly(*N*-isopropylacrylamide) (PNIPAAm, LCST range of 30–50°C), increasing the temperature over the PNIPAAm's LCST results in the formation of temperature-sensitive polymersomes

[60,70]. The thermosensitivity of polymers with LCST properties can also be used to trigger the drug release from nanovesicles. A triggered release is often achieved by decreasing the target tissue local temperature with external ice packs or with penetrating cryoprobes to deform the vesicles.

Polymersomes that respond to dual triggers (temperature and reducing agents) are also reported [57]. PEG-PAA-PNIPAM tri-block copolymers have been used as building blocks to form polymersomes. Elevating the temperature over the polymer's LCST results in the formation of nanocarriers in an aqueous environment. Crosslinking with cystamine locks the structures and creates polymersomes which are stable at human body temperature. However, these polymersomes are sensitive to a reductive environment due to cystamine's presence in the structure [57].

A newly developed, thermosensitive polymersome encapsulating another polymer (called a hydrosome) has been developed for sustained therapeutic delivery. The polymersomes are prepared from poly(ethyleneglycol)-*b*-poly(DL-lactide) block copolymers that encapsulate PNIPAAm at room temperature. When injecting polymersomes into the body, a phase transition for PNIPAAm (at temperatures higher than the LCST) leads to the formulation's instability and triggers the encapsulated drug's release [71].

Other stimuli-responsive polymersomes

Polymersomes that encapsulate magnetic nanoparticles have been utilized for the controlled delivery or release of drug/imaging agents using an exogenous magnetic field [54,72]. By encapsulating the magnetic nanoparticles in the hydrophilic core, the contents can be released at the target site by local hyperthermia [54] or by deforming the system with an external magnetic field [73]. The polymersomes can be guided to the target site magnetically. This property may be a promising approach to overcome obstacles with drug delivery across blood–brain barriers. Two of the most studied and promising magnetic nanoparticles incorporated into polymersomes are Fe_3O_4 and $\gamma\text{-Fe}_2\text{O}_3$, owing to their biocompatibility, biodegradability and stability [72,73]. Although these polymersome-based drug carriers have many promising properties, concerns regarding possible agglomeration and embolization inside the capillary vessels need to be addressed [72,73].

To render polymersomes photo-responsive, a photo-labile bond is introduced between the polymers' hydrophilic and hydrophobic blocks [53,58]. Exposure to UV light (365 nm) cleaves the bond, followed by polymersome deformation and shrinkage, leading to the triggered release of encapsulated drugs. The content release can be controlled by the intensity of the UV light used [58]. However, this mode of triggered content release from the polymersome is not devoid of problems. In the reported system, UV irradiation at a 365 nm wavelength is required for efficient release. This wavelength can harm healthy tissues.

Diagnostic-frequency ultrasound (1–10 MHz) is a noninvasive approach that is widely used for biomedical imaging [8,74]. Entrapping air bubbles in the nanocarriers endows echogenic properties, making these carriers good ultrasound contrast agents [8]. High-frequency ultrasound has been used extensively as a stimulus for triggered drug release from liposomes [75] and micelles [76]. However, ultrasound-induced drug release from polymersomes has

been unsuccessful [8]. The polymersomes' membrane thickness and high stability are likely responsible for this failure.

Overexpressed enzymes in a tumor environment are another trigger to release encapsulated drugs from nanocarriers at the targeted site. With this strategy, a substrate for the target enzyme is usually introduced in the nanocarrier. At the tumor tissue, peptide cleavage destabilizes the drug carrier's structure and releases the encapsulated contents.

Overexpressions of matrix metalloproteinases [77,78] (MMP-9 and MMP-2), serine proteases and cysteine protease [79] (Cathepsin B) in tumor tissues have been utilized for designing enzyme-responsive polymersomes [26] and liposomes [67].

Pharmacokinetic properties of polymersomes

The fate of the pharmaceutical agents after administration are determined by measuring their pharmacokinetic properties. However, polymersomes are new drug carriers, and not many detailed pharmacokinetic animal studies are reported (the reported results are summarized in Table 3). It should be noted that a drug's pharmacokinetic properties are related to the carrier chemistry. A comparison of the pharmacokinetic properties of PolyDoxoSomes (doxorubicin loaded polymersomes) with that of LipoDox (commercially available liposomal doxorubicin) [80] is reported. PolyDoxoSome's lower AUC (568 versus 229 $\mu\text{g h/ml}$), and shorter $t_{1/2}$ (22 versus 35 h) were advantageous for decreasing the dose related toxicities and side effects. Although the AUC and $t_{1/2}$ values were different, the therapeutic efficacy of the PolyDoxoSome was comparable to that of the LipoDox. Another study compared the pharmacokinetics of AmbiSome (liposomal amphotericin B) and amphotericin-B-loaded polymersomes [81]. A Lower AUC value was observed for the polymersomes (11,354 vs 62,219 ng h/ml), due to the direct delivery of the drugs to the target tissues. However, higher $t_{1/2}$ (74 versus 27 h) was reported for the polymersomes, due to the slow release of the encapsulated drugs. The polymersomal formulations were deemed to be better for treating fungal infections due to the longer circulation of these drug-loaded nanovesicles.

Hybrid lipo-polymersomes

Due to the increased stability, polymersomes are emerging as promising vesicles to replace liposomal formulations. However, several concerns remain for including polymersome-based drug-delivery systems in commercial formulations. The consequences of long-term administration and possible accumulation of the polymersomes in different organs are not known. Thus, polymersomes may be less biocompatible compared with liposomes. It appears that each formulation (i.e., liposomes and polymersomes) has its own benefits and limitations.

Hybrid lipopolymersomes (HLP) have been prepared to address the limitations of liposomes and polymersomes. These recently studied nanostructures (consisting of both polymers and lipids) not only have excellent robustness and tunable structural properties of polymersomal formulations but also retain the liposomes' biocompatibility and biofunctionality [84,85]. A recent study demonstrates that targeting cancer-cell receptors can be achieved more efficiently using HLPs compared with polymersomes [86].

Electroformation [52,84], film rehydration followed by extrusion [84,87], and particle fusion (liposomes and polymersomes) using salt and agitation [88] have been used to prepare HLPs. The major challenge with HLP preparation is the heterogeneity of the formed nanovesicles. Due to the inherent structural differences between lipids and polymers, the formation of mixed vesicles is possible (Figure 4). Depending on the amounts of polymers and lipids, lipid-rich domains in a polymer-rich membrane (Figure 4A), polymer-rich domains in lipid-rich membranes (like lipid rafts in cell membranes; Figure 4B) and membranes with homogenously distributed lipids and polymers (Figure 4C) are observed [52,84,87]. In extreme cases, phase separation is also possible, forming separate liposomes and polymersomes (Figure 4D). However, with high polymer-to-lipid ratios, homogenous HLPs are formed. There are some discrepancies for the composition and membrane characteristics of constructed HLPs, possibly due to differences in the fluidity of the lipids used to construct the vesicles. It appears that the nature of the constituents along with their molar ratios determine the nature of the formed vesicles. In addition, differences for the phase behaviors of giant vesicles ($> 1 \mu\text{m}$) and nanovesicles should be considered as a source of discrepancy in the resultant products [84].

Conclusion & future perspective

Considering the progress in developing polymersomes as theranostic nanovesicles, it is envisioned that polymersomes will play an important role as future pharmaceuticals. However, it should be noted that these formulations have some drawbacks. A low drug-loading efficiency, the presence of toxic, residual organic solvents in the final product, a lack of specificity toward the target site, and nonefficient control of the payload release need to be addressed. Therefore, future studies should focus on the preparation methods that are free of organic solvents, can be scaled up and possess efficient targeting, and release properties. Although many studies demonstrate the effectiveness of polymersomes in mice or rats, shifting toward species that are evolutionarily closer to humans may accelerate the translational processes. To increase selectivity, polymersomes are usually decorated with ligands for cell-surface receptors of target tissues. However, differences in human's genetic contents and a lack of expression for some receptors in different patients need to be considered [89]. Another important factor that warrants more attention is the polymersomes' pharmacokinetics. For long-term administration, the polymersomes' toxicity on vital organs needs to be studied.

Key terms

Solvent-exchange

With this procedure, block copolymers are dissolved in an organic solvent, and this solution is added to an aqueous solution while stirring. Solvent evaporation leads to the formation of polymersomes

Film rehydration

For this strategy, block copolymers are dissolved in an organic solvent, and the solvent is evaporated to form a thin film. Rehydrating the film leads to the formation of polymersomes

Electroformation	This method is useful when forming giant polymersomes (>1 μm). A volatile organic solvent, usually chloroform, is used to dissolve the polymers. Solvent evaporation leads to the formation of an ordered layer of polymers on the electrodes' surface. Hydrating the polymer film using an aqueous solvent with an applied, alternating current leads to the release of the formed polymersomes
Double-emulsion strategy	This polymersome-formation procedure dissolves polymers in a volatile organic solvent (oil), and the oil is used to form a double emulsion of water in oil in water. Dissolved amphiphilic, block copolymers form a monolayer at the oil-water interface, and by evaporating the solvent, the formation of polymersomes is achieved
Reticuloendothelial system	This part of the immune system (also known as the mononuclear phagocyte system) is responsible for filtering out foreign particles, bacteria, viruses and dead or abnormal cells

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Executive summary

What are polymersomes?

- Polymersomes are polymeric vesicles with an aqueous interior and an amphiphilic bilayer, making them a valuable delivery vehicle for single/combinational therapy. The polymersomes' hollow core can be loaded with hydrophilic theranostics, and the amphiphilic bilayer can be used to incorporate hydrophobic payloads.

Preparation methods for polymersomes

- Linear/Y-shaped, amphiphilic block copolymers are normally the building blocks of polymersomes.
- Linear, amphiphilic block copolymers tend to form polymersomes only if the copolymer has a certain ratio (25–40%) of hydrophilic part/total mass.
- The solvent-exchange method and film rehydration are the most common procedures to prepare polymersomes.

Polymersomes' biocompatibility

- Biocompatible polymersomes, ideally, do not react with blood components, and do not affect nontarget tissues.
- The most common procedure to make biocompatible polymersomes is using polyethyleneglycol (PEG) as the hydrophilic polymer. PEG is “generally regarded as safe” (GRAS) by the US FDA.
- Functionalization of polymersomes using targeting moieties (e.g., antibodies, peptides, small molecules and carbohydrates) is an effective approach to improve polymersomes' biocompatibility.

Polymersomes' biodegradability

- Ideal polymersomes should release the cargo at the target site in a controllable manner. Hence, the polymersomes' building blocks need to be responsive to external (light and magnetic field) or internal (redox potential, pH and temperature) stimuli.

Figure 1. Polymersomes

(A) Transmission electron microscopic image of a polymersome constructed using poly (ethyleneglycol)-*b*-poly (lactic acid) copolymer. (B) Schematic illustration of combination cancer therapy using a polymersome. The hydrophilic core of the polymersome is loaded by a conventional, hydrophilic, anticancer drug (Doxorubicin) and a hydrophobic chemotherapy drug (Paclitaxel) is incorporated in the hydrophobic bilayer of the polymersome.

Figure 2. Spherical/worm-like micelles and polymersomes

Changing the f value of the block copolymers determines the formation of different structures.

Figure 3. Schematic distribution of polymersomes after injection

(A) Stealth polymersomes can evade the reticuloendothelial system (RES) and accumulate in the tumor tissue as a result of the Enhanced Permeability and Retention effect. In this case, systemic toxicity decreases, and the drug's therapeutic index increases. (B) Nonstealth polymersomes can be recognized by the RES and cleared from the blood rapidly. Tumor accumulation is not lower than that for RES organs such as the liver and spleen. In this case, systemic toxicity is an important concern.

Figure 4. Different groups of hybrid lipopolyversomes

(A) Hybrid lipopolyversomes (HLPs) with lipid-rich domains in polymer rich membranes.

(B) HLPs with polymer-rich domains in lipid rich membranes. (C) HLPs with

homogenously distributed lipids and polymers. (D) Phase separation of the lipid-polymer mixture along with the formation of separate polymersomes and liposomes.

Table 1

Polymersomes for targeted theranostic delivery.

Targeting moiety	Target site	Polymersomes' building block	Drug/imaging agent loaded	Application	Ref.
Trastuzumab	HER2	Poly (trimethylene carbonate)- <i>b</i> -poly(glutamic acid)	Superparamagnetic iron oxide nanoparticles (USPIOs; γ Fe ₂ O ₃)	Targeting and imaging of breast-cancer bone metastasis	[25]
Anti- EGFR antibody	EGFR	mPEG-Gly-Phe-Leu-Gly-Phe-poly(D,L-lactide)	Acridine orange	Targeted drug delivery to the breast-cancer cells	[26]
Anti-ICAM-1 antibody	Intercellular Adhesion Molecule-1 (ICAM-1)	PEO- <i>b</i> -polybutadiene	–	Targeting the inflammatory tissues	[27]
Anti-HlgG antibody/Anti-HSA antibody	Human IgG/Human Serum Albumin (HSA)	PEG- <i>b</i> -poly(D,L- lactic acid)	Carboxyfluorescein	Theranostic targeting	[28]
Trastuzumab	HER2	Poly(dimethylsiloxane)- <i>b</i> -poly(2-methyloxazoline)	–	Theranostic delivery to the SKBR3 human breast-cancer cells	[29]
Anti-tansferrin receptor antibody (OX26)	Transferrin receptor	PEG- <i>b</i> -poly(ϵ -caprolactone)	NC-1900/Coumarin-6	Brain drug delivery	[30]
Tet1 peptide	Trisialoganglioside clostridial toxin receptor on neurons	Poly(ϵ -caprolactone)- <i>b</i> -PEG	Carboxy cyanine dye DiI	Drug delivery to the cochlear nerve	[31]
PR_b peptide	α 5 β 1 integrins	PEO- <i>b</i> -poly(butadiene)	TNF- α	Drug delivery to prostate cancer cells	[32]
RGD-containing peptide	Integrin adhesion receptors	PEO- <i>b</i> -poly(γ -methyl-3-caprolactone)	–	Cell penetration of theranostics	[33]
RGD-containing peptide	Integrin adhesion receptors	PEG- <i>b</i> -poly(2-hydroxyethyl aspartamide) substituted with octadecyl chains	FITC-dextran	Cell penetration of theranostics	[34]
Des-octanoyl ghrelin	Des-octanoyl ghrelin binding sites on BBBs	Poly(carboxyl ethylene glycol- γ -glutamate)- <i>co</i> -poly(distearin- γ -glutamate)	Doxorubicin/Cy5.5	BBB theranostic delivery	[35]
Folate and des-octanoyl ghrelin	Folate receptor & Des-octanoyl ghrelin binding sites on BBBs	Poly(ethylene glycol)- γ -glutamate)- <i>co</i> -poly (distearin- γ -glutamate)	Met-enkephalin/Coumarin-6/Cy5.5	Penetrating and targeting brain-cancer therapeutics	[36]
sLex and anti-ICAM-1 antibody	P/E selectin and ICAM-1	Poly(1,2 butadiene)- <i>b</i> -poly(ethylene oxide)	Porphyrin, PZn2	Monitoring inflammatory diseases	[1,37]
Lactoferrin/Transferrin	LRP/transferrin receptor	Poly (butadiene- <i>b</i> -ethylene oxide)+ PEG- <i>b</i> -lactic acid) (PEG-PLA)+ maleimide-PEG-PLA	Coumarin-6	Brain delivery	[38]
Lactoferrin	LRP	mPEG-poly(ϵ -caprolactone) + α -carboxyl poly(ethylene glycol)-poly(3-caprolactone)/PEG-poly (lactide)	Doxorubicin/Tetrandrine/Dir/Coumarin-6	BBB and glioma targeting	[39,40]

BBB: Blood–brain barrier; mPEG: Methoxy poly(ethylene glycol); PEG: Poly(ethylene oxide); RGD: Arg-Gly-Asp

Table 2

Biodegradable polymersomes.

Block copolymer	Description	Release stimulus	Response	Ref.
PEG-S-S-PLA	–	Redox potential	Bond cleavage	[8]
PEG-PTMBPEC	–	pH	Hydrolysis	[55]
mPEG-b-(polyHis) ₂ -CL	Cholesterol incorporated	pH	Hydrophobic/hydrophilic transition (structural transition)	[9]
PEG-PTTMA-PAA	–	pH	Hydrolysis	[56]
PEG-PAA PNIPAM	Cystamine-crosslinked	Temperature & reduction	Formation/deformation	[57]
PTMC-b-PGA	Polymersomes loaded with γ -Fe ₂ O ₃	Magnetic field	Local hyperthermia	[54]
PEG-2NPA-PLA	–	Light	Peptide bond cleavage	[53]
PMCL-ONB-PAA	–	Light	Cleavage of linker	[58,59]
mPEG-pep-PDLLA	–	Enzyme (Cathepsin B)	Peptide cleavage	[26]

mPEG-b-(polyHis)₂-CL: Methoxy poly(ethyleneglycol)-b-poly (L-histidine)-cholesterol incorporated; mPEG-pep-PDLLA: methoxy poly(ethyleneglycol)-Gly-Phe-Leu-Gly-Phe-poly (D,L-lactide); PEG-2NPA-PLA: Poly (ethyleneglycol)-2-nitrophenylalanine-poly (lactic acid); PEG-PAA-PNIPAM: Poly(ethyleneglycol)-b-poly (acrylic acid)-b-poly (*N*-isopropylacrylamide); PEG-S-S-PLA: Poly (ethylene glycol)-b-poly (lactic acid); PEG-PTMBPEC: Poly(ethyleneglycol)-b-poly (2,4,6-trimethoxybenzylidenepentaerythritol carbonate); PEG-PTTMA-PAA: poly(ethyleneglycol)-b-poly(trimethoxybenzylidene tris(hydroxymethyl)ethane methacrylate)-b-poly (acrylic acid); PMCL-ONB-PAA: poly (methyl caprolactone)-o-nitrobenzyl-poly (acrylic acid); PTMC-b-PGA: Poly(trimethylene carbonate)-block-poly (L-glutamic acid).

Table 3

Pharmacokinetic properties of polymersomal formulations.

Polymersomes building blocks	Drug	Dose (mg/kg)	AUC	Free drug AUC	Drug $t_{1/2}$ (h)	Free drug $t_{1/2}$ (h)	Animal	Ref.
Polybutadiene (PBD)-b-PEG	NA	0.42 of polymersomes	NA	NA	24	NA	Male Balb/C mice	[82]
PEG-b-poly(3-caprolactone)(PCL)	Oxymatrine	30 of drug	30,602 $\pm$ 3982 ng h/ml	16,234 $\pm$ 1385 ng h/ml	3.12 $\pm$ 0.25 [†]	1.41 $\pm$ 0.57 [†]	Male SD rats	[83]
PEG-PLA	Doxorubicin	6 of drug	569 μ g h/m	4 μ g h/ml	21.9	0.49	Female SD rats	[80]
PBD-PEO:PEG-PLA:MAL-PEG-PLA Lactoferrin conjugated	NA	20 of polymersomes	219.5 $\pm$ 25.6 mg h/l	NA	6.49	NA	Mice	[38]
PBD-PEO:PEG-PLA:MAL-PEG-PLA Transferrin conjugated	NA	20 of polymersomes	404 $\pm$ 49 mg h/l	NA	13.6	NA	Mice	[38]
PEG-PLA	Amphotericin B	1 of drug	11,354 ng h/ml	NA	73.84*	NA	Male SD rats	[81]

[†] Terminal phase half life.

NA: Not available; SD: Sprague-Dawley.