Biodegradable Polymer Vesicles: Design and Performance as Drug Delivery Carriers

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Drug delivery system (DDS) is used to administer a pharmaceutical compound to achieve an optimal therapeutic effect. This technology involves improvement of therapeutic index, control of drug release rate, minimization of drug degradation and reduction of drug toxicity in the body offering means of optimizing therapy with established drugs. Basically, numerous types of carriers can be used providing a variety in design and development of advanced DDS. As a new class of the polymer-based carriers, polymersomes (Ps) have attracted rapidly growing interest. A number of Ps has been developed for new possibilities and applications in drug delivery, medical imaging, electronics and nanoreactors. In particular, biodegradable and/or stimuli-sensitive block copolymers can be used for preparation of Ps, which are of great interest for such applications. In this review, recent advances of Ps and the performance as drug delivery carriers are discussed. Critical factors in the design and preparation of Ps are also addressed.

Key words: Polymersomes, Block copolymer, Controlled drug release, Circulation kinetics and biodistribution

Introduction

Recently, Ps have attracted tremendous attention as versatile carriers. Ps are artificial vesicles made from amphiphilic block copolymers. Typical polymeric vesicles contain an aqueous solution in the core surrounded by a bi-layer membrane. The bi-layer membrane is consisting of hydrated hydrophilic coronas both at the inside and outside of hydrophobic part of the membrane (Figure 1). The membrane can integrate hydrophobic drugs within its hydrophobic core. The aqueous core can be utilized for the encapsulation of therapeutic molecules such as drugs, enzymes, other proteins and peptides, and DNA and RNA fragments. The possibility to load drugs into Ps has been highlighted for a number of applications in medicine, pharmacy, and biotechnology.

Ps can be rather stable due to the relatively thick membranes formed by amphiphilic block copolymers with a relatively high molecular weight. The composition and molecular weight of the building polymers can be varied, which allows the preparation of Ps with different membrane properties such as permeability and rate of degradation. In particular, stimuli-responsive Ps has attracted a lot of interest to further control the release of drugs by switching the stability and permeability of the membrane. Ps based on block copolymers that are responsive to pH, temperature, redox conditions, light, magnetic field, ionic strength and concentration of glucose have been reported.

The presence of a hydrophilic PEG brush on the surface will reduce the protein adsorption onto the Ps during the blood circulation. Carriers with a PEG brush on the surface are generally considered to have “stealth character” due to minimization of the interfacial free energy and the steric repulsion provided by the presence of a hydrophilic PEG brush on the surface.
the PEG molecules. For site-specific drug delivery, it is also important to guide Ps to the specific target area and to enhance their interaction with specific cells in this area. This can be achieved by introducing targeting moieties, for example, antibodies, antibody fragments, or RGD-containing peptides on the surface of the Ps. End groups of the PEG can be used to immobilize homing moieties like antibodies or RGD-containing peptides, which are able to recognize target cells or tissues. These Ps can release drugs by external stimuli after arrival at the target site enhancing the therapeutic efficacy and minimizing possible side effects. In order to design such Ps, it is necessary to understand the requirements for the polymers to be used for the formation of Ps. In this review, critical factors in design of Ps and their performance as drug delivery systems will be given.

**Formation of Polymersomes**

**Preparation methods**

There are many techniques, which can be used to prepare Ps by self-assembly of amphiphilic block copolymers. Typical methods are polymer rehydration techniques, which are based on the hydration of amphiphilic block copolymer films to induce self-assembly. Polymers are first dissolved in an organic solvent and then a thin film is prepared by evaporation of the organic solvent. Subsequently, the film is hydrated by the addition of water. The steps in the formation of Ps by the hydration procedure are water permeation through defects in the polymer layers driven by hydration forces, inflation of polymer layers and formation of bulges, which finally yield vesicles upon separation from the surface. This method produces Ps with a broad size distribution and therefore the Ps obtained are subsequently sized by sequential extrusion through filters with different pore sizes using a high pressure. Other important preparation methods are solvent-switching techniques and polymer rehydration techniques. Using the solvent-switch technique, Ps are formed by first dissolving block copolymers in an organic solvent after which the organic solvent and then a thin film is prepared by evaporation of the blocks present, followed by hydration of the solution. This procedure renders the hydrophobic blocks insoluble, triggering copolymer self-assembly into Ps as a result of increasing interfacial tension between the hydrophobic blocks and water. Block copolymers of which the hydrophobic blocks have a high glass transition temperature (Tg) cannot directly form Ps by using the polymer rehydration method. An organic solvent has to be used to lower the Tg to provide sufficient chain mobility.

**Requirements of block copolymers as building materials of polymersomes**

In principle, amphiphilic block copolymers can self-assemble into a wide range of morphologies upon hydration of the copolymer including spherical, cylindrical micelles or vesicles. In the classical description, the packing parameter (p) is one factor to presume the morphologies. The p is related to the curvature of the hydrophobic-hydrophilic interface as described by its mean curvature (H) and its Gaussian curvature (K). Different morphologies are corresponding to different values of p, for instance, p < 1/3 (spheres), 1/3 ≤ p ≤ 1/2 (cylinders) and 1/2 < p ≤ 1 (vesicles). The mass or volume fraction of the hydrophilic block of the block copolymer (f) and the interaction parameter of its hydrophobic block with H2O (χ) are known to be other critical parameters to determine the morphology of the self-assembled system. For Ps based block copolymers with a high γ, vesicular structures are favored when f of PEG (fPEG) is 10-40%. At fPEG = 45-55%, cylindrical micelles tend to form, and at fPEG = 55-70%, spherical micelles are predominantly formed. Vesicular formation can also be influenced by the preparation methods and conditions like polymer concentration, the type of organic solvent and the volume ratio of solvent and water.

**Drug Loading and Release from Polymersomes**

**Drug encapsulation**

The membrane of Ps can be considered as a reservoir system for both hydrophobic and amphiphilic molecules similar to cell membranes, which incorporate cholesterol and membrane proteins. It has been reported that highly lipophilic anticancer drugs, dyes and quantum dots as well as amphiphilic dyes (i.e. octadecyl rhodamine B) and membrane proteins (i.e. OmpF, LamB and FluA) can be integrated within the membrane of Ps while maintaining their functionality. These molecules can be incorporated in Ps by first dissolving or dispersing them together with the membrane-forming polymer building blocks in an organic solvent after which the organic solution dispersion is added to water or an aqueous solution. In this way, paclitaxel (PTX) or doxorubicin (DOX) could be loaded into the membranes of Ps with comparable loading amounts and efficiencies as compared to other self-assembled carriers. Several methods are currently used for the loading of hydrophilic molecules, but the most common methods are direct encapsulation during formation of Ps or diffusive loading methods using a pH or salt gradient over the membrane of already formed Ps.

**Modulated drug release from polymersomes**

In principle, drug release from Ps is governed by the diffusion of the drug through the membrane. The driving force is a concentration gradient of the drug between Ps and the sur...
Drugs from the Ps. Hydrogels can be introduced in Ps to modulate the release of copolymers are of interest. On the other hand, stimuli-sensitive comonomers or simple blends of different degradable block copolymers with a hydrophobic block consisting of the rate of degradation and consequently drug release. Either degradable polymers may be very challenging to further control since each biodegradable polymer has a unique hydrolysis rate in contact with water or enzymes. Biodegradable block copolymers based on PLA, PCL and PTMC, and hydrophilic blocks like PEG have already been used to prepare biodegradable Ps. Ps with membranes based on different biodegradable polymers may be very challenging to further control the rate of degradation and consequently drug release. Either block copolymers with a hydrophobic block consisting of comonomers or simple blends of different degradable block copolymers are of interest. On the other hand, stimuli-sensitive hydrogels can be introduced in Ps to modulate the release of drugs from the Ps. Polymers, which are sensitive to various stimuli (i.e. temperature, pH and etc) can be encapsulated with drugs or proteins in Ps and this may change the morphology of the interior of the Ps. Hydrogels in the Ps can form by external stimuli and will influence the diffusion rate of drugs from the interior of the Ps to the surroundings.

**Biodegradable Polymersomes with tunable membrane permeability**

Novel approaches to control the release of drugs from Ps are to use different biodegradable polymer compositions to prepare Ps or to modify the interior of the Ps. By selecting different biodegradable polymers, the permeability of the Ps membrane can be varied and the release of drugs from the Ps may be controlled since each biodegradable polymer has a unique hydrolysis rate in contact with water or enzymes. Biodegradable block copolymers based on PLA, PCL and PTMC, and hydrophilic blocks like PEG have already been used to prepare biodegradable Ps. Ps with membranes based on different biodegradable polymers may be very challenging to further control the rate of degradation and consequently drug release.

Biodegradable or non-biodegradable Ps have been developed for delivery of PTX controlled by using amphiphilic block copolymers with different hydrophobic blocks. Ps based on block copolymers such as PEG-PBD, PEG-b-poly(epsilon-caprolactone) (PEG-PEE), PEG-b-poly(lactic acid) (PEG-PLA) and PEG-PCL were prepared. The latter two copolymers are biodegradable and it was shown that the mechanical properties and the rate of degradation of these Ps membranes depend on the character and length of the hydrophobic blocks. Two amphiphilic di-block copolymers, monomethoxy PEG-b-poly (D,L-lactide) (mPEG-PDLLA) and mPEG-b-poly(epsilon-caprolactone) (mPEG-PCL) were also used to prepare different types of drug-loaded Ps. Ps were prepared by injecting THF solutions of mPEG-PDLLA, mPEG-PCL or a mixture of the block copolymers (50:50, w/v) with or without a model drug for PTX into PBS or DI water. Ps made of mPEG-PDLLA, mPEG-PCL and a mixture of two block copolymers have been abbreviated as Ps (L), Ps (C) and Ps (LC), respectively. Fluorescent paclitaxel (Flutax) was used as a model drug for PTX to study the release kinetics for four types of Ps formulations.

Release of Flutax from suspensions of Ps placed in a microdialysis system was monitored by periodic withdrawal of PBS samples (Figure 2). A sustained release of Flutax was observed for Flutax-Ps (L) and complete release of the drug was accomplished after one month. Ps (C) released 49.9% of the loaded Flutax over 1 month, which is much lower than the release from Ps (L). This can possibly be related to the crystallinity and the rate of degradation of the consisting copolymers. Ps (LC) showed similar release kinetics as Ps (L+C), but the release rate became slightly lower after 1 week. This may be due to the fact that a relatively slow release of Flutax is governed by PCL domains in the later stages, whereas a relatively fast release of Flutax from the PDLLA domains (probably located at the surface of the hydrophobic part of the membranes) occurs in the early stages. Notably, Ps formations using different combinations of various biodegradable and/or non-degradable block copolymers would be of interest to further control the release kinetics.

**Polymersomes containing thermosensitive hydrogels**

Stimuli-sensitive hydrogels can be introduced in Ps to modulate the release of drugs from the Ps. Polymers, which are sensitive to various stimuli (i.e. temperature, pH and etc) can be encapsulated with drugs or proteins in Ps and this may

![Figure 2](image-url)
change the morphology of the interior of the Ps. Hydrogels in the Ps can form by external stimuli and will influence the diffusion rate of drugs from the interior of the Ps to the surroundings. Poly(N-isopropylacrylamide) (PNIPAAm) hydrogel-containing polymersomes have been previously reported. The hydrogel-containing Ps (Hs, hydrosomes) were prepared by a solution of PNIPAAm and mPEG-PDLLA into DI water (Figure 3a). Above the low critical solution temperature (LCST) of the PNIPAAm solution in the Ps, phase separation takes place, and a hydrogel and an aqueous phase are formed (Figure 3b). Fluorescence correlation spectroscopy and fluorescence anisotropy measurements with these systems gave evidence for the colocalization of PNIPAAm and Ps. The release of fluorescein isothiocyanate tagged dextran (FD, FITC-dextran, Mw 4,000 g/mol) from Hs revealed that in the presence of the hydrogel at 37°C a more sustained release of FD (up to 30 days) with a low initial burst effect was obtained as compared to the release from bare Ps (Figure 3c). These results were explained by the formation of a membrane associated PNIPAAm hydrogel layer in the Ps, which strongly reduces the release rate of FD.

Stimuli-responsive polymersomes

The physical and chemical properties of some Ps membranes are changeable in response to external stimuli. Stimuli-responsive Ps as programmable delivery systems have recently attracted rapidly growing interest. Significant efforts have been devoted to develop Ps which are sensitive to stimuli like pH, temperature, redox potential, light, magnetic fields and ultrasound. Some of these stimuli are able to trigger the disintegration of Ps for instance by a change in the hydrophilic/hydrophobic properties of the block copolymers or by poration of the membrane as a result of the preferred cleavage of covalent bonds in the polymer chains of one polymer component of the membrane. These possibilities in changing the properties of Ps by external stimuli are promising for the controlled release of drugs from the Ps after arrival at the target site, where the stimulus is present. In this way, the efficacy of the drugs at the site of action can be enhanced and side effects reduced.

The use of enzymes for targeted drug delivery has also been recognized as a very interesting approach. For instance, lysosomal enzymes like cathepsin B (Cath B) are expressed at increased levels in tumor tissue as compared to normal tissue. These enzymes can be used to cleave certain peptide sequences like the tetrapeptide sequence, Gly-Phe-Leu-Gly (GFLG). Structures containing this peptide sequence can be transformed by the cleavage of the peptide, resulting in the release of loaded or covalently bound drug. For example, N-(2-hydroxypropyl) methacrylamide (HPMA) copolymer-DOX conjugates with GFLG linkers were produced in the early 1980s. Application of the water soluble conjugates containing GFLG in a mouse melanoma tumor model resulted in concentrations of approximately 70 times more DOX in the tumors than in normal tissues. Ps based on a biodegradable and biocompatible block copolymer of mPEG and PDLLA in which a peptide sequence, Gly-Phe-Leu-Gly-Phe (GFLGF), was introduced in between the two blocks (mPEG-pep-PDLLA) were also developed. Aggregation and precipitation of the peptide containing Ps by disintegration of the Ps were observed as a result of cleavage of the peptide. The rate of disintegration of the Ps was depending on the concentration of Cath B and the pH. Acidine orange (AO) was encapsulated in the Ps as a model drug and rapid release of AO triggered by Cath B was observed at pH 5.5.

Blood Clearance and Biodistribution of Polymersomes

Circulation kinetics of polymersomes - comparison with stealth liposomes

Although there is little known about the opsonization process of nanocarriers due to the complexity of the biological events,
surface coating, charge and size of nanocarriers are undoubtedly playing important roles in the blood clearance.\textsuperscript{30-33} Opsonization processes can be influenced by the variation of MW and surface concentration of PEG molecules. The effect of PEG on the circulation time of Ps based on PEG-PBD with different MW was investigated in rats.\textsuperscript{39} Ps with a PEG of MW 2300 g/mol exhibited a half lifetime of $28 \pm 10$ h, while a half lifetime of $15.8 \pm 2.2$ h was obtained when PEG with a MW of 1200 g/mol was used. Stealth liposomes coated with PEG (MW 1900 g/mol) (7.5-10 mol%) had shorter half lifetimes of 10-15 h in rats when compared to Ps with a similar MW of PEG. It has been suggested that the surface of Ps may adsorb less and/or different plasma proteins due to a higher surface concentration of PEG as compared to the liposomes.

**Biodistribution of polymersomes**

Ps are known to accumulate primarily in the liver.\textsuperscript{38,39,50} Adsorption of liver specific opsonins probably enhances the uptake of Ps by liver macrophages, Kupffer cells and this process may play a major role in the hepatic uptake of the vesicles.\textsuperscript{53} Interactions with the opsonins can be reduced by introduction of a slightly negative or positive charge on the surface of Ps, yielding prolonged blood circulation times.\textsuperscript{54,55} Likewise, a range of optimal sizes for specific nanocarriers has been suggested to establish long circulation times (e.g. stealth liposomes with diameters from 70 to 200 nm).

Ps based on poly(ethylene glycol)-b-poly(D,L-lactide) (PEG-PDLLA) with similar sizes, but different zeta potentials ($-7.6$ to $-38.7$ mV) were prepared to investigate the effect of surface charge on blood circulation time and tissue distribution in tumor-bearing mice as compared to stealth liposomes (Figure 4).\textsuperscript{38} PEG-PDLLA polymersomes with a low zeta potential ($-7.6$ mV) and a diameter of approximately 100 nm had a much longer half lifetime and a reduced liver uptake (28% ID after 3 d) as compared to stealth liposomes. It may possibly be conclusive that the effects of the charge density of anionic polymersomes on circulation kinetics and biodistribution showed that polymersomes with a slightly negative surface charge are most suited for in vivo administration.

**Conclusions and Perspectives**

Polymeric vesicles are capable of encapsulating hydrophilic, hydrophobic and amphiphilic molecules like any other vesicular structure, but their thick and robust membrane provides them with superior stability. The presence of a dense PEG brush with relatively long PEG polymers on the surface of polymersomes may increase their biological stability and prolong the circulation times in blood. Polymersomes are versatile systems and their overall properties and drug release profiles can be easily tuned by applying various block copolymers that are possibly biodegradable and/or stimuli-responsive. However, it should be noted that the PEG coating can also diminish the uptake of these carriers by cells since the PEG brush reduces cell-carrier interactions. Therefore, it would be interesting to design novel stimuli-responsive Ps that are provided with biologically active homing devices as transport vesicles for drugs to further increase the concentration of drugs at specific target sites. This can be achieved by introducing targeting moieties, for example, antibodies, antibody fragments, or RGD-containing peptides on the surface of the Ps.

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