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Abstract

Polymersomes are self-assembled polymer shells composed of block copolymer amphiphiles. These synthetic amphiphiles have amphiphilicity similar to lipids, but they have much larger molecular weights, so for this reason—along with others reviewed here—comparisons of polymersomes with viral capsids composed of large polypeptide chains are highly appropriate. We summarize the wide range of polymers used to make polymersomes along with descriptions of physical properties such as stability and permeability. We also elaborate on emerging studies of in vivo stealthiness, programmed disassembly for controlled release, targeting in vitro, and tumor-shrinkage in vivo. Comparisons of polymersomes with viral capsids are shown to encompass and inspire many aspects of current designs.

INTRODUCTION

Viral capsids self-assemble from viral polypeptides to protect the viral genome within a robust shell and also to integrate mechanisms for viral targeting and controlled release. Polymersomes are self-assembled from synthetic polymers—rather than natural polymers—and are now being engineered to perform some of the same functions as robust viral capsids, i.e., carry, target, and release actives (drugs and dyes). Since their inception less than a decade ago, polymersomes have been compared more with lipid vesicles than with viral capsids, but viral capsids increasingly make a better comparison on the basis of what has been learned about the properties achievable with polymersomes. As reviewed here, the choice of synthetic polymer(s) as well as the choice of molecular weight (MW) of the polymer is important as these are particularly distinctive molecular features that impart polymersomes with a broad range of tunable carrier properties. Additionally, a range of relevant polymers, such as polyethylene glycol-polylactic acid (PEG-PLA) and PEG-polycaprolactone (PEG-PCL), are commercially available off-the-shelf or custom synthesized (from Polymer Source Inc., Montreal, and Sigma Chemicals, St. Louis); therefore, broadened accessibility to material motivates an up-to-date review.

The polymers used to make polymersomes are similar to lipids in that they are amphiphiles: At least one fraction or "block" of each molecule is water loving or hydrophilic, whereas the other fraction or block is hydrophobic. Either type of amphiphile, if made with suitable amphiphilic proportions, can self-assemble into vesicles when hydrated. The hydrophobic blocks of each molecule tend to associate with each other to minimize direct exposure to water, whereas the more hydrophilic blocks face inner and outer hydrating solutions and thereby delimit the two interfaces of a typical bilayer membrane. Lipids are of course derived from nature and tend to be biocompatible—a fact that has partially motivated their development into liposomal drug carriers. Such efforts have often been synergistic with a host of studies aimed at more basic understanding of the nature of cell membranes. Although liposomes lack controlled release mechanisms and possess a number of other limitations, including major pharmacokinetic shortcomings, liposomes are often cited as the dominant nanocarriers today [estimated at $0.5 billion per year (P. Cullis, personal communication)]. One should keep in mind that, similar to viral capsids, carriers are intended to merely influence delivery and not be active in and of themselves. It is therefore perhaps revealing in the present context that one of the major chemotherapeutic formulations with liposomes, DOXIL® (from Alza Co.), which encapsulates the water-soluble drug doxorubicin, attaches biocompatible PEG to a significant fraction of its lipids.

Lipids typically have a total MW of less than 1 kDa, whereas natural polypeptides or proteins have an order of magnitude MW of 10 kDa, corresponding to approximately 100 amino acids. The low MW of lipids imparts biomembranes with a lateral fluidity and additional "soft" properties (1) that appear conducive to various cellular processes (endocytosis, cell division, receptor clustering, etc.). In contrast, polymersomes are composed of synthetic polymers of MWs more typical of polypeptides, which again suggests closer analogies between polymersomes and viral capsids. One

goal of this brief review is to elaborate on the current repertoire of synthetic polymers making up polymersomes, illustrating the wide range of possible chemistries—especially biocompatible chains. A second goal is to describe the influence of polymer choice, especially MW, on membrane thickness and properties. The results to date suggest fertile ground for mimickry of viral capsids as well as some promise for a wide variety of tailored applications.

POLYMERSOME STRUCTURE AND PROPERTIES

Self-Directed Assembly of Polymer Vesicles

Principles underlying self-directed assembly of natural amphiphiles are general and revealing (2). Lipids and many small amphiphiles differ considerably in their hydrophilic fraction or "head group," but they often contain one or more strongly hydrophobic chains composed of multiple ethylene units $(\text{-CH}_2\text{-CH}_2\text{-})_n$ (with $n = 5$ to 18 typically). A simple solution thermodynamic measure of aggregate stability is provided in terms of n by the critical micelle concentration

$$C_{\text{CMC}} \sim \exp(-n\varepsilon_h/k_b\text{T}), \tag{1}$$

where k_bT is the thermal energy, and ε_h is the monomer's effective interaction energy with the bulk solution (related to χ in polymer physics). Only at surfactant concentrations above C_{CMC} do aggregates such as vesicles form. For ethylene groups and biological temperatures $\varepsilon_h \sim 1$–$2\ k_b T_{\text{biol}}$ (~4–8 pN nm) so that values of C_{CMC} for natural lipids and related amphiphiles in aqueous solutions range from micromolar to picomolar (i.e., aggregates are stable in high dilution). Amphiphile exchange rates between aggregates are also generally proportional to C_{CMC}, with characteristic exchange times for phospholipids estimated in hours. Owing to the exponential dependence on n or hydrophobic MW in Equation 1, polymer-based amphiphiles with large hydrophobic MW_h (compared with lipids) generally lead to highly stable aggregates, as well as some degree of kinetic trapping.

Block copolymers have the same amphiphilic character as lipids but consist of polymer chains covalently linked as a series of two or more blocks (**Figure 1*a***). In the absence of any solvent, block copolymers are known to display a wide range of ordered morphologies, including lamellar phases (with the same symmetry as a stack of paper). Hydration, even in the presence of a block-selective solvent such as dioxane, will swell initially dry lamellae and fluidize the layers, even if a glassy polymer such as polystyrene is used in the diblock copolymer such as with polystyrene-polyacrylic acid (PS-PAA); the end result of hydration into a volume is a stable dispersion of vesicles (3). Most of the polymer vesicles reviewed below exploit less glassy copolymers than PS and largely eliminate the need for fluidizing cosolvent even though polymer MWs are still far greater than those of natural lipids.

One of the earliest examples of a diblock that self-assembles directly under solvent-free aqueous conditions is a dipeptide construct PS_{40}-poly (isocyano-L-alanine-L-alanine)$_m$ (4). It is semisynthetic in the sense that it contains naturally occurring peptide moieties. Under acidic conditions and for $m = 10$, collapsed vesicular shells

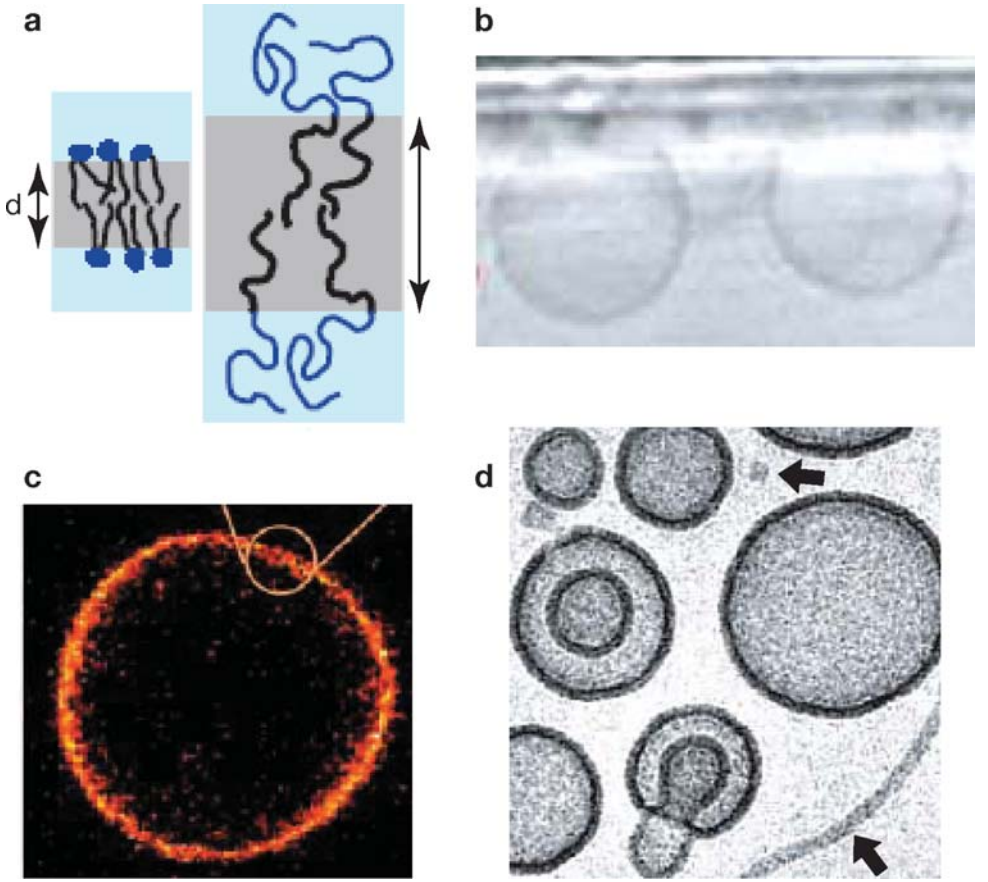

Figure 1

(*a*) Natural lipid versus synthetic polymer assemblies. (*b*) Self-directed assembly of polymersomes from hydrated films. (*c*) Fluoro-polymersome. (*d*) Cryogenic transmission electron microscopy of ~100-nm polymersomes. The two arrows point to spherical and rod-like micelles that sometimes coexist with polymersomes.

with diameters ranging from tens to hundreds of nanometers were observed coexisting with rod-like filaments and chiral superhelices. With the use of fully synthetic diblock copolymers of PEO_m–PBD_n (PEO, polyethylene oxide; PBD, polybutadiene) and the hydrogenated homologue of PBD, poly(ethylethylene) (yielding PEO-PEE), unilamellar polymer vesicles first referred to as polymersomes were made under a variety of aqueous conditions (5). **Figure 1*b*–*d*** shows how the addition of water to a microns-thick lamellar film of PEO-PBD generates micron-diameter polymersomes that can be (*a*) labeled with fluorescent dyes as fluoro-polymersomes, as first used in studies of lateral mobility (6), or (*b*) sonicated, film-squeezed, or extruded to make 100-nm (virus-size) vesicles, as used in cell-culture and in vivo studies (7, 8).

f Dictates Aggregate Morphology Whereas MW Dictates Thickness

The effective interaction energy ε between hydrophobic monomers in polymer blocks is key to aggregate stability (Equation 1), whereas the relative mass or volume fraction of each block is key to morphology. Vesicles are closely related to rod-like and spherical micelle morphologies (**Figure 2*a***) in that they are solvent-dependent, self-directed assemblies. Micelles have been widely reported for many lipid-size surfactants (2), as well as much larger polymeric superamphiphiles (9, 10). However, micelles lack a shell-like character and will not encapsulate a bulk solution phase—including hydrophilic drugs, proteins, nucleic acids—as a vesicle or a virus can.

For a simple amphiphile in aqueous solution, a time-average molecular shape in the form of a cylinder, wedge, or cone (**Figure 2*a***) dictates whether membrane, cylindrical, or spherical morphologies will respectively form (2, 14). This average molecular shape is most simply a reflection of the hydrophilic fraction *f*. Illustrative of such geometric forces at work is the behavior of lipids when conjugated at the hydrophilic head group with PEG chains (equivalent to PEO). With DOXIL and many related liposomes, the typical PEG chain used [MW ~ 2000–5000 Da (11)] leads to a conically shaped amphiphile distinct in form from cylindrically shaped phospholipids and with $f \gg 50\%$. Consequently, phospholipid membranes mixed with PEG-lipid at more than 5%–10% mole fraction tend to generate highly curved micelles. Despite this membrane-disruptive tendency of PEG-lipid at high concentrations, low concentrations appear compatible with vesicle morphology and technologically useful. Upon injection into the circulation, these so-called stealth pegylated liposomes are cleared more slowly from the blood than conventional liposomes (12, 13). As a result of the extended circulation time, stealth vesicles loaded with anticancer drugs such as doxorubicin circulate long enough to find their way into well-hidden but permeable tumors.

Polymers such as PEG are clearly useful for biomedical application and raise the question as to whether a vesicle-type system composed entirely of polymer will work as well. Although we discuss below that polymersomes can indeed circulate as long or longer than stealth liposomes, the systematics of making polymer vesicles in water needs to be studied first. On the basis of the examples above, along with others, one unifying rule (or at least a starting point) for generating polymersomes in water is a phospholipid-like ratio of hydrophilic to total mass: $f \approx 35\% \pm 10\%$. A cylindrically shaped molecule that is asymmetric with $f < 50\%$ presumably reflects the ability of hydration to balance a disproportionately large hydrophobic fraction. Molecules with $f > 45\%$ can be expected to form micelles, whereas molecules with $f < 25\%$ can be expected to form inverted microstructures.

Sensitivities of the rules above for chain chemistry and MW have not been exhaustively probed. However, copolymers following these rules and yielding polymersomes have thus far had average MWs ranging from ~2000–20,000 Da. Cryogenic transmission electron microscopy of 100–200-nm vesicles further shows that the membrane core *d* increases with MW from $d \approx 8$–21 nm (**Figure 2*b***) (15, 16). Lipid membranes have a far more limited range of $d \approx 3$–5 nm, which is clearly compatible with the

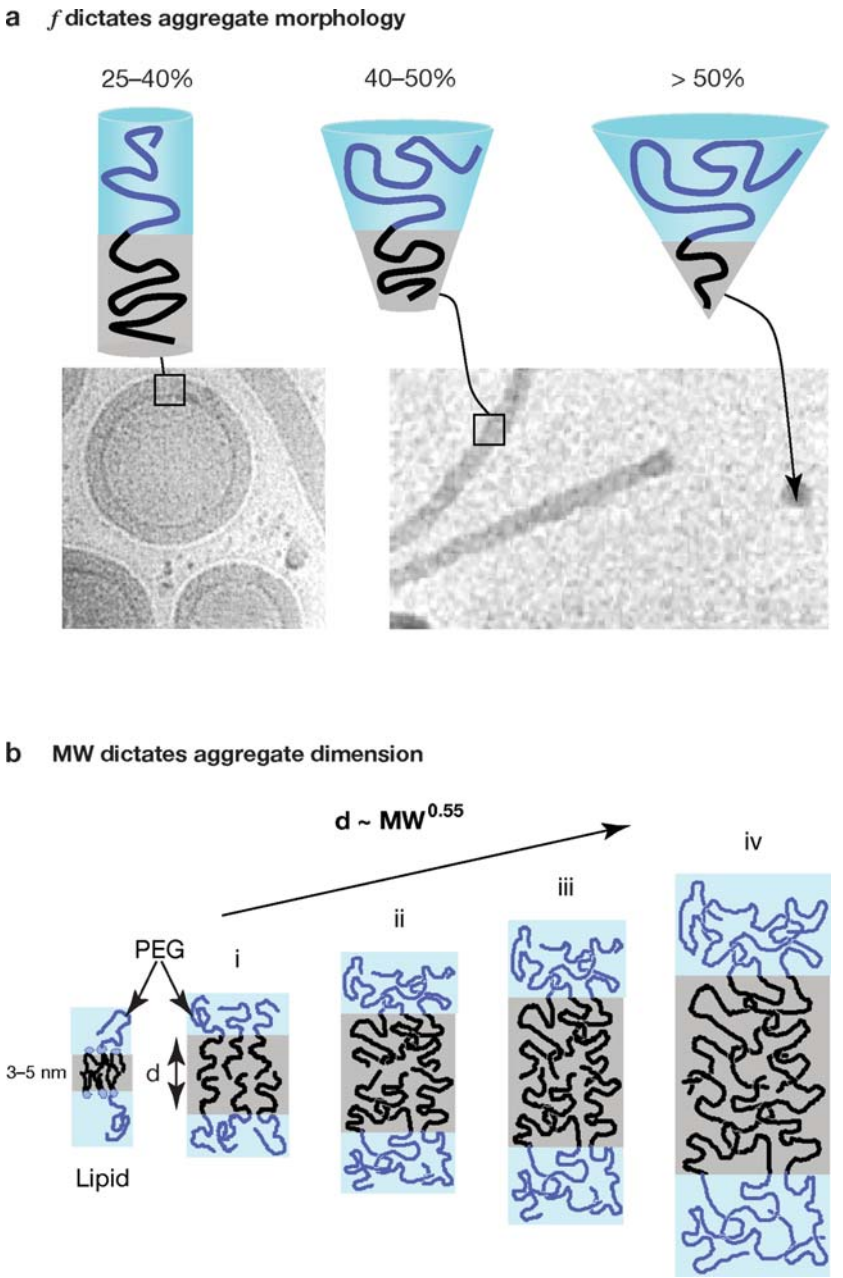

Figure 2

(*a*) Schematics of block copolymer fractions with respective cryogenic transmission electron microscopy images showing vesicles or worm micelles and spherical micelles. (*b*) Schematic scaling of polymersome membrane thickness with copolymer molecular weight (MW). PEG, polyethylene glycol.

many integral membrane proteins in cells. Scaling of copolymer membrane thickness with copolymer MW was experimentally documented and confirmed by a novel coarse-grained molecular dynamics simulation that faithfully captures atomistics (17) leading to

$$d \sim MW_{\mathrm{h}}^{\mathrm{b}} \ (b \cong 0.55). \tag{2}$$

Perhaps most revealing from simulation has been that, for low MW systems, the polymer bilayers have a clear midplane of low density, whereas high MW copolymers show two bilayers that interdigitate or "melt" together into a single thick shell of homogeneous density. Polymersome membranes thus present a novel opportunity to study membrane properties as a function of membrane thickness *d*.

Polymersome Fluidity, Stability, and Other Properties

Micron-size "giant" polymersomes allow for detailed characterization of membrane properties by single-vesicle micromanipulation methods (5). Measurements of lateral diffusivity (6) as well as apparent membrane viscosity (18, 19) have shown that membrane fluidity generally decreases with increasing MW (**Figure 3**), and the fluidity decreases most drastically when the chains are sufficiently long to entangle. Area elasticity measurements for PEO-PBD membranes provide an indirect measure of ε_{h} as γ(~30 mN/m), which appear independent of MW as expected. This indicates that opposition to interface dilation is dictated by polymer chemistry and solvent alone. Electromechanical stability also increases with membrane thickness up to a limit set by γ (15). Phospholipid membranes rupture well below such limits (e.g., rupture tension scaled by γ) simply because their small *d* makes them more susceptible to defects. Lastly, permeation of water through the polymersome membranes shows, in comparison with phospholipid membranes, a considerably reduced transport rate. This is consistent with early measurements on liposomes by Bangham (20), the pioneer of

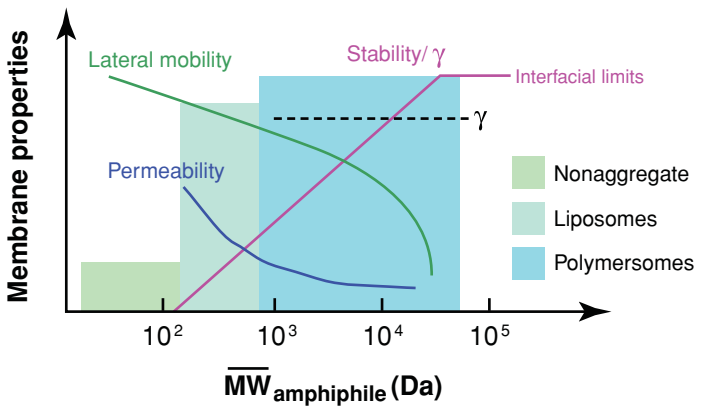

Figure 3

Schematic plot of typical physical properties of vesicles versus molecular weight of the constituent amphiphiles.

liposomes, on a relatively narrow MW series of lipids. What appears most suggestive from the physicochemical studies to date is that lipid membranes have evolved in nature to be optimized more for fluidity than for stability. Conversely, robustness, solidity, and low permeability are all hallmarks of viral capsids, which also possess additional intrinsic features such as programmed disassembly for controlled release.

POLYMERSOMES FOR DRUG DELIVERY

Viruses have evolved over millenia (and continue to evolve) to release their nucleic acid at a suitable time and suitable place. Disassembly of a viral capsid is sometimes triggered at low pH and is often sensitive to environmental variables such as temperature. In comparison, many if not all conventional liposome systems have proven to be both inherently leaky (11) and short-lived in the circulation (21). To integrate mechanisms of controlled release, PEG-lipids have been made to play dual roles as liposome stabilizers that also, upon exposure to an environmental stimulus, effectively destabilize the carrier membrane via thiolytic (22) or hydrolytic (23, 24) cleavage. Block copolymers seem to broaden the possibilities and perhaps sharpen the transitions.

Hydrolysis and Other Triggers of Release from Polymersomes

Block copolymers of PEO-polylactide (PEO-PLA is equivalent to PEG-PLA) have been known in the past to make micelles and nanoparticles (25, 26) and have been of great interest because PLA is susceptible to hydrolytic biodegradation, which should foster drug release (27). However, vesicles made from PEG-PLA had not been reported years ago probably because of the narrow range of f required for a stable vesicle phase (**Figure 2*a***). Indeed, the molecular-scale force balance outlined in the sections above allows the design of PEG-block-based copolymers that—in the absence of degradation—form membranes in preference to other structures. Solid-like particles of PEG-PLA had been reported with diblock copolymers having small hydrophilic PEG fractions of $f < 20\%$ and large MW hydrophobic blocks (25, 28), whereas for $f > 42\%$, both worm micelles (up to $f \sim 50\%$) (29, 30) and spherical micelles of PEG-PLA (31) and poly(ethylene glycol)-poly(caprolactone) (PEG-PCL) (32) had been reported. With $f \cong 20\%$–42% for these two types of block copolymers, "loose" micellar architectures have been reported (31), but such a description seems apt for the degraded remnants of vesicles first shown on the cover of the August 9, 2002, issue of *Science* and soon thereafter characterized (33, 34).

Polyester-based degradable polymersomes (**Figure 4*a***) have thus far been made from PEG-PLA or PEG-PCL and while they do make stable vesicles (34), they have been studied most thoroughly as blends with inert PEO-PBD to protract the degradation and release times. Indeed, the release rates of encapsulated molecules increase linearly with the mole ratio of PEG-PLA (34), and it is visibly clear that the membranes are homogenous, as verified by fluorescence microscopy of mixed PEG-PLA/PEO-PBD membranes (33). Blends with lipids are also possible, in principle: A moderate MW triblock copolymer will mix homogenously with lipids in

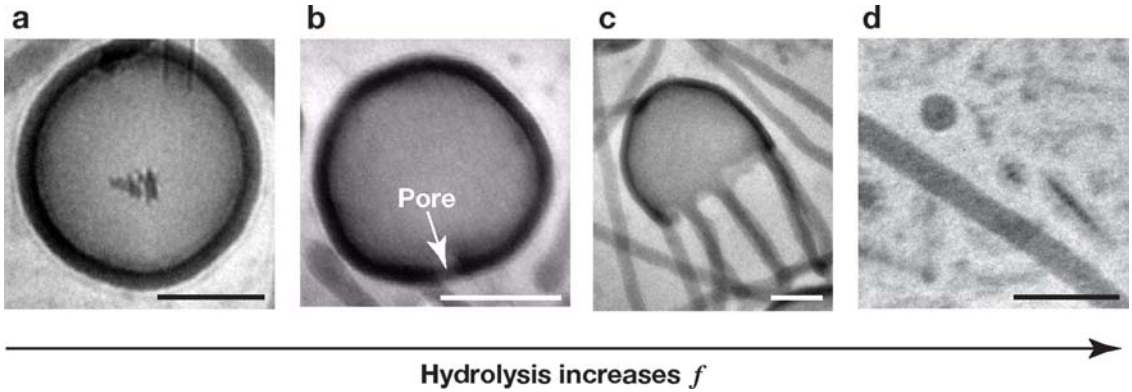

Figure 4

Typical time series of breakdown structures of PEG-PLA nanopolymersomes, starting with vesicles and ending in worm micelles. Scale bars are 100 nm.

polymer-lipid composites (35). This is surprising to some extent because even slight differences in lipid chain length can cause microphase separation.

Polyester hydrolysis occurs preferentially at the chain end (30), so the resultant curvature preference of PEG-polyester chains in the membrane of a vesicle transforms the stable bilayer-forming chain into a detergent-like copolymer. Such degraded chains with comparatively short hydrophobic blocks will tend to segregate, congregate, and ultimately induce hydrophilic (i.e., PEG-lined) pores (**Figure 4*b***). Encapsulant release is controlled through blends on timescales ranging from hours to weeks. These molecular scale transitions are evident in various physical observations, such as encapsulant release that decreases with increasing MW. Eventually, these vesicle carriers disintegrate into mixed micellar assemblies—as is evident by both fluorescence microscopy and cryogenic transmission electron microscopy measurements (**Figure 4*c*,*d***). Block polyester participation in the bilayer morphology appears strongly dependent on the rate of hydrolysis of the hydrophobic block (e.g., PCL versus PLA), as well as the hydrophilic block ratio, f. Parallel studies with varied polyester hydrophilic/hydrophobic block ratios, hydrophobic chemistry, and different mole-percent blends indicate that polyester chain hydrolysis is indeed the molecular trigger controlling encapsulant release and polymersome carrier destabilization kinetics. Ongoing studies demonstrate a strong dependence of degradation rate on pH, as recently reported for degradation of worm micelles composed of PEO-PCL (30).

Instead of hydrolyzing one polymer block, Hubbell and coworkers (36) have developed oxidation-responsive polymersomes from PEO-(propylene sulphide)-PEO triblock copolymers. The idea is to exploit the oxidative environment present in sites of inflammation as well as within endolysosomes. Studies to date show that exposure to either aqueous H_2O_2 or H_2O_2 from a glucose-oxidase/glucose/oxygen system will oxidize the hydrophobic propylene sulphide core and transform it to poly(sulfoxides) and poly(sulfones), which are hydrophilic. Oxidation thus increases f of the

macroamphiphile and thereby destabilizes the vesicle morphology, eventually leading to soluble oxidized copolymer.

Charged Polymersomes

Although charged liposomes tend to be cleared quickly from the circulation (21, 37), the addition of charge to polymer amphiphiles presents additional opportunities for controlled release in response to external stimuli, such as pH [e.g., PAA-mediated endolysosomal rupture (38)]. However, the shape and structure of charged diblock copolymer aggregates in solution are governed by an especially delicate balance of forces. Interfacial tension between the core and the bulk solvent continues to orient, confine, and stretch the chains, but the interactions between solvated corona blocks can be repulsive, through sterics and electrostatics, as well as attractive if multivalent ions are involved (2). Eisenberg and coworkers (10) mapped out the first morphological phase diagram of charged diblock copolymers in dilute solution for "crew-cut" PAA-PS copolymers. The rich phase behavior and dynamics of a relatively symmetric PAA-PBD diblock have now also been mapped out in water (39). Depending on the concentration of added calcium or sodium salt as well as pH, these diblocks are seen to self-assemble in water into stable vesicles, worms, and spheres. Furthermore, using fluorescence microscopy, researchers visualized transitions of vesicles into worms and spheres within minutes with a sudden increase of pH or chelation of salt. Liu & Eisenberg (40) have likewise shown rapid pH-triggered inversion of biamphiphilic triblock copolymer vesicles of PAA-PS-poly(4-vinyl pyridine) in organic/aqueous mixtures. With increasing bulk pH, the aggregate morphology changes from vesicles, with poly(4-vinyl pyridine) outside, to solid aggregates and then inverts back into a whole vesicle assembly, with PAA on the outside. Some of these design principles seem likely to make their way into biocompatible polymersomes and certainly inform us about the dramatic effects of charge in polymer systems.

Peptide-Based Vesicles

A rapidly emerging approach to polymer-based vesicles involves a return to biology with peptide-based assemblies. In contrast to viral capsids with lock-and-key assemblies of great precision, the peptide-based vesicle assemblies have amphiphilicity as their foundation. Emerging results from several groups suggest there is no need for nature's lock-and-key approach, in principle. For example, PBD-(poly-*L*-glutamic acid) diblock copolymers self-assemble in aqueous solution into vesicles (peptosomes) (41) in which the hydrophilic polypeptide segment forms a well-defined secondary structure of α-helix. In dilute aqueous solutions, the copolymers form either spherical micelles or larger vesicular aggregates. In addition, these systems exhibit pH- and ionic-strength-dependent changes in hydrodynamic radius and in coil-helix transitions. In addition to providing the hydrophobic driving forces for self-assembly, the PBD block can be laterally cross-linked (33, 42); this and related cross-linking (43) covalently capture the aggregate in a shape-persistent nanoparticle (44). Such nanoparticles may be suitable for applications such as encapsulation or release of

hydrophilic and hydrophobic active species or in sensor nanodevices, where the latter systems could take advantage of protein channels that—perhaps surprisingly—will integrate into the hyperthick block copolymer membranes (45, 46).

Hybrid block copolymers constructed from amphiphilic β-strand peptide sequences and PEG have recently been studied (47). In comparison with the native peptide sequence, di- and triblock copolymers with PEG were shown to stabilize the peptide's secondary structure against pH variation and to self-assemble into helical tapes that stack into fibrils. Vesicles have not been reported but seem feasible by this approach. Conversely, diblock copolypeptides of {poly(N_e-2-(2-(2-methoxyethoxy)ethoxy)acetyl-*L*-lysine}–(poly-*L*-leucine) have been shown to self-assemble into bilayer vesicles whose size and structure are dictated primarily by the ordered conformations of the peptide segments (48), in a manner similar to viral capsid assembly. If 70% of the *L*-leucine in the hydrophobic domain is replaced in a statistical manner with *L*-lysine, the system becomes pH sensitive. At pH 9, vesicles form, but protonation of the lysine residues enhances their hydrophilicity and destabilizes the α-helical structure of the leucine-rich domain owing to electrostatic repulsion of the like charges. These helix-to-coil transitions destabilize the vesicular assembly, leading to porous membranes or complete disassociation of the structures. Recent extensions of polymer-peptide hybrids include the attachment of hydrophobic polymers such as PS to enzymes with the subsequent self-assembly into spherical aggregates and vesicles that still retain biological activity (49, 50). Proteins are also, of course, being encapsulated within polymer vesicles, and the latest work includes polymersome-encapsulated hemoglobin, which yields oxygen affinities comparable with that of human red blood cells (51).

Polymersome Interactions with Lipid Membranes

Self-assembled polymer systems can, with prudent design, open new pathways for the delivery of drugs, similar in principle to how viruses deliver their nucleic acid within cells. Cationic aggregates are a popular, sometimes toxic, choice of carrier that fosters intracellular delivery through a "proton sponge" effect on internalizing vesicles (52). However, these charged nanoparticles are cleared from circulation within minutes (53). We have recently attempted to address the problem of endosomal escape with neutral degradable polymersomes. We find that under mildly acidic conditions found in endolysosomes, PEG-polyester polymersomes degrade, generating polymeric macrosurfactants that actively interact and disrupt model lipid membranes (54). The results might resolve a controversy over escape mechanisms with PEO-PCL micelles (55) because the accumulated data suggest that the lytic time constant τ_{lysis} for PEG-polyester-mediated rupture of lipid membranes scales inversely with hydrophilic fraction *f* while scaling directly with MW of the hydrophobic block (**Figure 5**). Simulations (17) mentioned above in the context of membrane-thickness scaling (Equation 2) have been extended to studies of lipid interactions and tend to confirm a lytic tendency with increasing *f*. Thus, although self-assembled membranes of copolymer and self-assembled membranes of lipid interact little, similar copolymers assembled into spherical micelles will fuse, mix, and porate a lipid membrane.

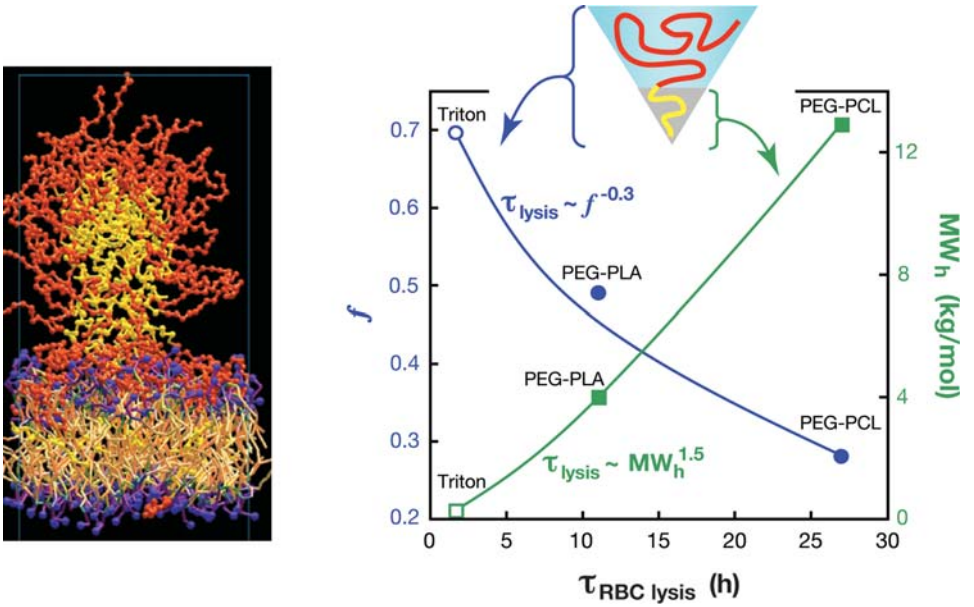

Figure 5

Polymer-lipid interactions. Plot (*right panel*) shows the relationship between polymer *f*, molecular weight (MW), and the time constant to the disruption of lipid membranes. PEG-PCL, polyethylene glycol-polycaprolactone.

If the micelles are generated by polymersome degradation, the same process applies. Given that the endosomal membrane is the cell's last barrier to cytoplasmic entry, the copolymer-mediated poration seen here clearly creates a new pathway for drug escape and delivery to cellular targets.

Polymersomes In Vivo

Liposomes have, of course, been widely investigated as circulating drug carriers, and PEG-lipid has been known for more than a decade to delay vesicle clearance (13). However, stable anchorage of the massive, hydrophilic PEG chain can be limited: Incubations in serum had shown that these liposomes immediately lose approximately one third of their PEG-lipids, presumably owing to facilitated micellization (56). As summarized above, polymersome architectures are founded on more proportional amphiphilic polymers and hence have more stable anchorage. Moreover, the far greater hydrophilic PEG (100%) content confers resistance to plasma protein deposition (opsonization) and further extends vesicle circulation times. As a result, the dense and stable PEG brush minimizes the deposition of plasma proteins (opsonization) and prolongs in vivo circulation (8). The plot in **Figure 6** documents the long circulation of first-generation polymersomes in rats, with half-lives of 20–30 h that significantly exceed the circulation times of stealth liposomes

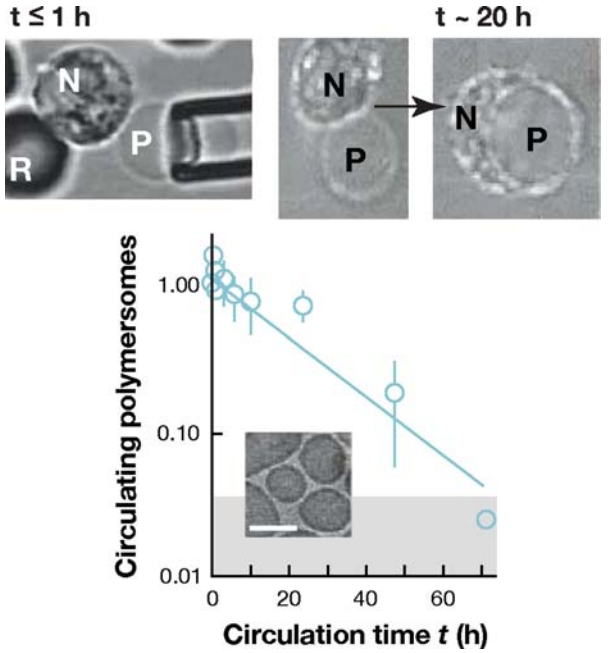

Figure 6

Prolonged circulation in rats of fluoro-polymersomes (~100-nm size) with details given in Reference 8. Top images show that at short times in serum (*left*), polymersomes (P) avoid clearance by phagocytic neutrophils (N) but are then engulfed (*right*) after many hours in serum. R, red blood cell. The glass micropipette in the top left image is approximately 3 microns in diameter, and the scale bar on the lower image is approximately 100 nm. The gray area of the graph is below the resolution of measurements.

in low-dose injections. The inset images in the figure further illustrate how the PEG brush of polymersomes limits adhesive and stimulating interactions with white blood cells for the first hours of suspension in blood; however, after 10 or more hours, the PEG brush no longer prevents protein adsorption, so the white blood cells are able to adhere and even engulf giant polymer vesicles. Similar cells in the liver and spleen appear responsible for the clearance of polymersomes from the circulation.

Active Targeting of Polymersomes

Although few if any drug carriers in clinical use today are targeted, viruses often show preferential—although not exclusive—interactions with particular cell types and cell entry pathways. Given that polymersomes exhibit minimal nonspecific adhesion to cells (initially), that they can circulate for many tens of hours, and that they can integrate controlled release mechanisms, targeted polymersomes should add an additional level of viral mimickry. With PEG-based assemblies, much work has been focused on attaching ligands or antibodies to the hydroxyl end-group (57, 58).

Biotinylation of nondegradable PEG-PBD diblocks (59) has allowed block copolymer assemblies to attach to (*a*) surfaces coated with the biotin receptor avidin as well as to (*b*) cells where they successfully delivered the hydrophobic cytotoxic drug Taxol®. Similar chemistry has been used to attach either an antihuman IgG or antihuman serum to PEG-carbonate- or PEG-polyester-assembled polymer vesicles (60). Meier and coworkers (61) have modified their triblock copolymer polymersomes with polyguancylic acid to target a macrophage scavenger receptor SRA1. This scavenger receptor is a pattern-recognition antigen upregulated only in activated tissue macrophages, not in monocytes or monocyte precursor cells. Though promising, these in vitro cell targeting studies have not been translated to in vivo conditions, where opsonization by serum components and competitive interactions with other cells often impede and complicate targeting efforts (58). Moreover, targeting via attached chemical groups—even if it were to provide a major advantage and justify the added complexity from a regulatory standpoint—does not act like a "magnet" with action at a distance; convective and/or diffusive collisions of the carrier with a cell must occur for targeted adhesion to take place. Retention of a multivalent adhesion of the carrier on the cell surface can then foster uptake or localized release, but if a carrier localizes within a tissue or tumor where convective and/or diffusive forces are minimal, then such retention offers little additional advantage. This is the essence of the enhanced permeation and retention (EPR) effect that is typical of solid tumors and other tissues (62). The EPR effect thus motivates studies of passively targeted carriers that localize at the intended site, enter cells perhaps, and release drug before being transported away.

Putting Viral Mimics to Good Use: Shrinking Tumors with Polymersomes

Based on many of the concepts above, loading, delivery, and cytosolic uptake of drug mixtures from degradable polymersomes have recently been shown to exploit both the thick membrane of block copolymer vesicles and their aqueous lumen, as well as pH-triggered release within endolysosomes. Taxol is a hydrophobic drug that has been loaded into the membrane (analogous to the dyes of **Figure 1c**), and—as described in the first studies of degradable polymersomes (34)—doxorubicin has been a main hydrophilic drug loaded into the vesicle lumen. These are two of the most common anticancer drugs used in the clinic. The initial in vivo studies demonstrate vesicle localization to tumors as well as growth arrest and shrinkage of rapidly growing tumors in nude mice after a single intravenous injection of polymersomes composed of (Polyethylene-glycol, PEG)-(Polyester) (54). The vesicles have also been shown to break down in vitro into membrane-lytic micelles within hours at 37°C and low pH, although storage at 4°C allows retention of drug for over a month. Cell entry of the polymersomes into endolysosomes subsequently leads to copolymer-induced endolysosomal rupture with release of cytotoxic drugs. The in vitro and in vivo data all generally fit to a simple two-step model of localization followed by degradation-coupled cytotoxicity. This first polymersome-based therapeutic may be just the first of many more to come.

CONCLUSIONS

Polymersomes broaden considerably the choice of vesicle chemistries and self-assembled structures. Polymersome properties such as high stability and low fluidity warrant analogies with viral capsids and provide further motivation for engineering in controlled release mechanisms as well as targeting groups. Incorporation of biomolecules as well as a broad spectrum of functionality suggests these synthetic carrier systems offer a truly generic approach for drug delivery.

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