Hydrodynamics and physics of soft objects/Hydrodynamique et physique des objets mous

Synthetic cell elements from block copolymers – hydrodynamic aspects

Éléments de cellules synthétiques provenant de copolymères en bloc

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Abstract

Amphiphilic block copolymers can self-assemble in water into various stable morphologies which resemble key cell structures, notably filaments and membranes. Filamentous 'worms' of copolymer, microns-long, are briefly introduced, and related dynamics of copolymer vesicle 'polymersomes' are reviewed. Fluorescence visualization of single worms stretched under flow demonstrates their stability as well as a means to control conformation. Polymersome membranes have been more thoroughly studied, especially copolymer molecular weight effects. We summarize results suggestive of a transition from Rouse-like behavior to entangled chains. Viewed together, the results ask the question: what physics are needed next to mimic cell activities such as crawling? ***To cite this article: P. Dalhaimer et al., C. R. Physique 4 (2003).***
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Résumé

Les copolymères en bloc amphiphiles peuvent s'auto-assembler dans l'eau selon différentes morphologies, ressemblant à des éléments structuraux clefs de la cellule, notamment les filaments et les membranes. Les « vers » (worms) filamenteux, de la taille du micron, seront brièvement évoqués, puis nous résumerons les propriétés dynamiques des vésicules de copolymers (polymersomes). La visualisation à l'aide de la microscopie à fluorescence de vers seuls, étirés dans un flux, démontre d'une part, leur stabilité et d'autre part, présente un moyen de contrôler leur conformation. Les membranes des polymersomes ont été étudiées en détail, et plus particulièrement les effets de la masse moléculaire du copolymère. Au final, nous résumerons plusieurs résultats qui suggèrent une transition d'un comportement de « rouse-like » à une chaîne enchevêtrée. Une vue d'ensemble des résultats permet de poser une question à savoir quelle est la physique nécessaire pour imiter des activités de la cellule tel que le « rampement » (cell crawling). ***Pour citer cet article : P. Dalhaimer et al., C. R. Physique 4 (2003).***
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1. Introduction

Block copolymers have long been known capable of forming ordered phases of various symmetries, including periodically arrayed rods and lamellae [1]. Work on similar, dilute phase microstructures has been more recent. Vesicles referred to as polymersomes, for example, form in aqueous solution from amphiphilic block copolymers, when suitably proportioned in their hydrophilicity [2]. Of course, synthetic vesicles are mimics of biological cell structures, which in many ways, can be viewed as more complex self-assemblies of lipids, proteins and other biomolecules in water. Such a perspective serves to highlight questions about the minimal physical basis for how a cell works.

Whether one is theorizing about collective responses of a cell or a synthetic structure such as a polymersome, fluidity and hydrodynamics clearly have important roles. Cell crawling on a substrate is a good example and begins to illustrate some of the relevant dynamical issues. Fig. 1 shows the roughened outline of a cell as it displaces during crawling. Of course, cells are microscale objects ($\sim$10 µm), which predisposes cellular processes to be low Reynolds number, Re [3]. Directed or convected motions are also generally slow and of low Re given typical cell crawling speeds of only $v \sim$ µm/min. Cell motions ought therefore to be augmented by molecular diffusion as generally set by D ($>$ µm^2/sec) in aqueous solution. However, while the drag on the crawling cell due to the aqueous external media is minimal [4] and diffusion is rapid outside a cell, the effectively high viscosity within a cell [4] tends to impede disorder. This high, internal viscosity arises in large part from sub-cellular structures such as polymerized cytoskeletal filaments (see Fig. 1). Such structures also have central roles in driving the cell forward. One prevailing hypothesis for cell crawling is that preferential polymerization at the membrane proximal end of a filament locally rectifies nano-scale Brownian fluctuations of the soft cell membrane [5]. Except for such living polymerization processes, filamentous and membrane cell structures with tunable cell-like hydrodynamic responses are now within synthetic reach, as illustrated by the worms and polymersomes described below.
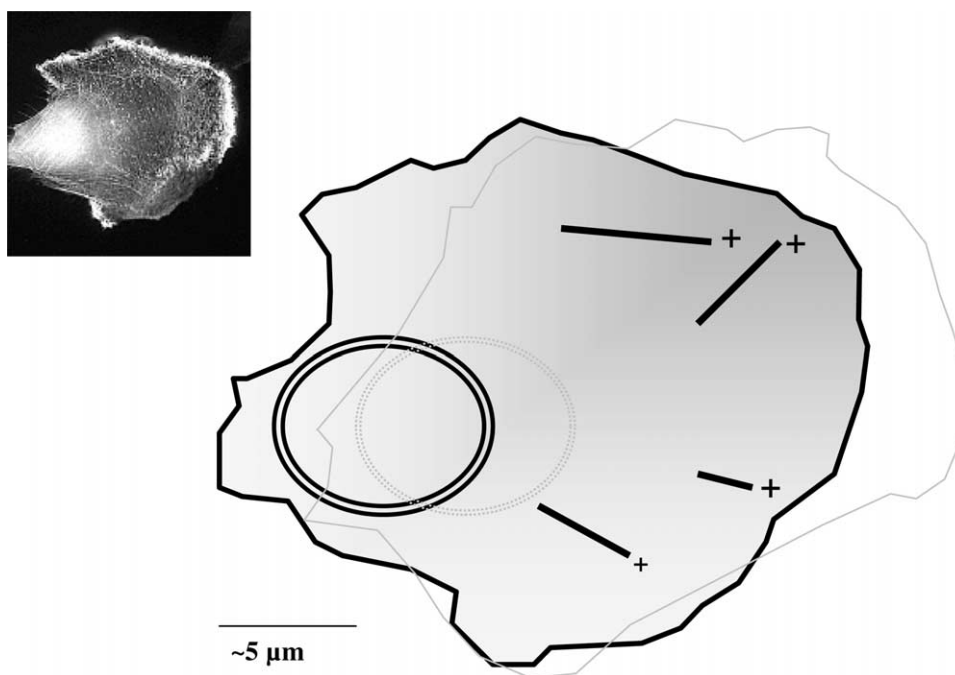

Fig. 1. Inspiration for synthetic polymer membranes and rods: cell crawling. Outlines of a cell traced from images of an actin-labeled cell crawling on an adhesive surface (inset). The cell's plasma membrane and inner nuclear (double) membrane are both displaced from left to right over a period of $\sim$1 min. Forward movement occurs primarily by polymerization of stiff actin filaments which are selectively shown as black rods or as dense gray regions. Polymerization is faster at the biased, (+)-ends of filaments. Anchoring of the opposite ends of the filaments extends through the membrane in cell adhesion to the substrate. Because the filaments are stiff, filament polymerization drives the membrane forward. Because the cell membrane is robust, it can be torn away from the substrate at the rear of the cell.

2. Giant worm-micelles and vesicles from block copolymers

Since Bangham first isolated lipid and reconstituted it into vesicles or liposomes [6], tremendous progress has been made in terms of both integrating biomolecules into the membranes [7] or encapsulating biomolecules that function within vesicles [8]. The latter cited reference is particularly notable as it demonstrates entrapment of the polymerizable protein tubulin in lipid vesicles and vividly shows that work on the membrane can be exerted by relatively stiff microtubules. Like cell crawling, which the system models in primitive form, the study again illustrates a synergistic complementarity between filaments and membranes. These systems are not particularly robust, however. Proteins generally denature over time and many phospholipid vesicles become leaky. Fully synthetic systems offer promise of simplicity and robustness. Beyond a fascination with structural mimicry, motivation is also found in a long term, unrealized goal of engineering synthetic cells that move in response to stimuli. The results here with membranes and worms begin to raise questions of physical principles requisite for a soft, synthetic 'living' system.

Block copolymer amphiphiles that assemble into either worm-micelles or closely related polymer membranes have very similar molecular structures. Fig. 2 illustrates the basic architecture of a two segment, diblock copolymer with a hydrophilic polyethyleneoxide (PEO) block and a hydrocarbon-based hydrophobic block. By making the fraction of the PEO block ~25–42%, bulk copolymer added to water will lead to the spontaneous formation of vesicles or polymersomes. As currently understood, hydration of the PEO generates sufficient osmotic force when aggregated with other diblocks to balance the hydrophobic volume of the remainder of the chain. However, with a relatively small increase in the PEO fraction to ~42–50%, hydration of the PEO overcompensates and induces a curvature in the aggregate. In either case, aggregate formation is strongly driven by the relatively high molecular weight of the hydrocarbon segment. This creates an interfacial tension, which separates the core from PEO as well as the bulk aqueous phase.

Considerable theoretical work has been done in the past on block copolymer mesophases and their elasticity (e.g., [9]), as well as on brushes [10]. Consistent with the strong segregation mechanism of aggregate formation above, it is already clear from measurements on vesicles that the interfacial tension, γ, is independent of molecular weight as well as relatively small changes in hydrocarbon chemistry [11]. However, relatively little appears to have been done on isolated versions of these microstructures such as worms and vesicles, especially in terms of hydrodynamic responses.

In phase contrast microscopy, giant polymersomes generally appear as diverse in shape as lipid vesicles [12]. Starfish, tube, pear (Fig. 2), and string-of-pearl shapes [2,13] are all prominent and in part reflect a relatively well understood vesicle-scale imbalance between the number of molecules in one leaflet of a membrane bilayer versus the number in the other leaflet. In other words, the membranes of these polymer vesicles appear to be bilayers, although the exact partitioning of amphiphiles between leaflets and the location of the midplane is not yet well controlled or understood. Nonetheless, cryo-transmission electron microscopy (cryo-TEM) is also consistent with a bilayer in that it shows a membrane thickness, d, which not only tends to be
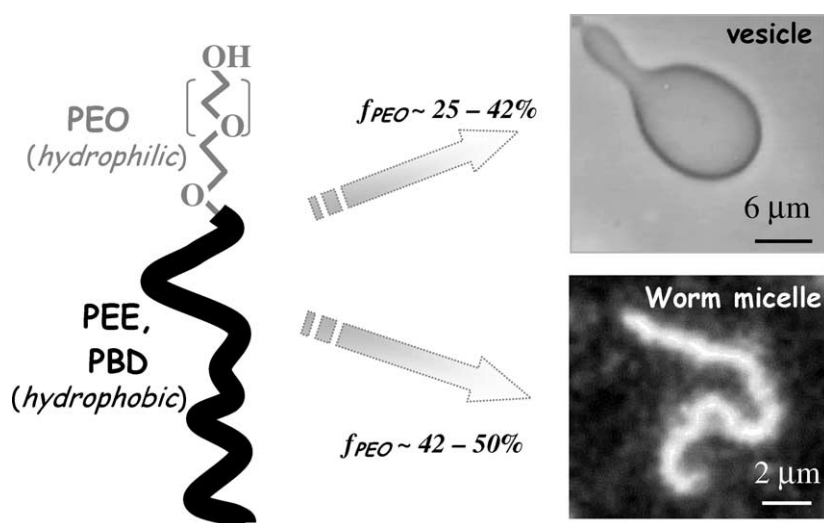

Fig. 2. Synthetic polymer membranes and worms made by self-assembly of PEO-based diblocks in water. The weight or volume fraction of the hydrophilic PEO (polyethylene oxide) block is specified by f. The hydrophobic, hydrocarbon block of the copolymers thus far studied consists of either PEE (polyethylethylene) or its crosslinkable analog PBD (polybutadiene). Note that an increased f by just a few percent leads to worm micelles instead of vesicles. Cryo-TEM has already shown that the worm micelles, made of block copolymers of molecular weight MW ~ 4 kDa, have hydrophobic cores of ~10 nm [20].

significantly greater than the 3–4 nm thickness of lipid bilayers but is also too thick for a single, convoluted copolymer to fully span [2].

Although the average shapes of polymersomes appear similar to liposomes, it is also evident that a given vesicle membrane undergoes minimal thermally excited bending fluctuations of the type which have been so thoroughly studied with both lipid vesicles [14] and cells [15]. As described further below, the membrane bending resistance is expected to increase with polymer molecular weight, *MW*, through membrane thickness, *d*. Additional properties, especially vesicle stability and in-plane hydrodynamic properties will also be shown to depend strongly and non-linearly on copolymer *MW*.

Worm micelles assembled by hydration and dilution of copolymers of ∼4 kg/mol have also been visualized: fluorescent labeling of the core with a hydrophobic fluorophore (PKH26; Sigma Chemical) and confinement to ∼1 µm high chambers shows worms up to 30 µm long. They appear highly flexible and dynamically contorted (see snapshot in Fig. 2). A diameter of about 10 nm for these worms is far less than optical resolution ($\lambda \sim 500$ nm) but the obviously large persistence length, l_P, of the worms means that the contour is almost always well visualized. This is unlike DNA [16] which has a $l_P \ll \lambda$ as well as much more complexity in terms of charge and chirality.

Despite having a similar diameter as the polymersome membrane thickness, the Brownian dynamics of worm micelles are highly pronounced in contrast to the membranes. Autocorrelation analyses of the easily identified end-to-end distance yields relaxation times of seconds for the micron long worms. Since these are fluid assemblies, as more fully indicated below for the polymersomes, it is an interesting but unanswered question as to whether the relaxation time reflects a dominance of dissipation in the worm or in the aqueous bulk. The stability of the worms is clear, however, and appears fully consistent with the high γ that drives membrane formation and underlies the stability of polymersomes.

3. Stiffness-tuned worm-micelles

In a flow field rather than under quiescent conditions, the fluid worms respond in a way roughly in agreement with present theory for polymers under flow [17]. Stuck occasionally at a single point to a coverslip, the worms increasingly straighten under high flow but their thermally accessed conformational space is always narrowest near the point of attachment (Fig. 3(A)). At the free end, these trumpet shapes extend up away from the coverslip but project down into an ellipse of fluctuations that is clearly much larger than the microscopic point of attachment and also dependent on the mean flow velocity as well as the length of the worm. Simple observation as well as more careful analyses of fluctuation modes [18] under quiescent conditions indicate a sub-micron persistence length, l_P, in agreement with estimations from neutron scattering [19]. The fact that the worms themselves are fluid and the imposed flow should slip rather than stick as a boundary condition does not appear obvious in these responses. It is clear, however, that the worms can withstand very high flow fields which can be estimated to impose tensions <1 µN/m calculated from a plane Poiseuille flow model with a velocity profile $v_x(y) = 3v[1 - (y/H)^2]$, where v is the average flow velocity, y is the distance between cover slips, and H is the gap height. The tension is the shear stress ($\mu \partial v_x / \partial y$; μ is the viscosity) integrated over the contour length of each worm. This places a lower bound on the γ holding the worms together.
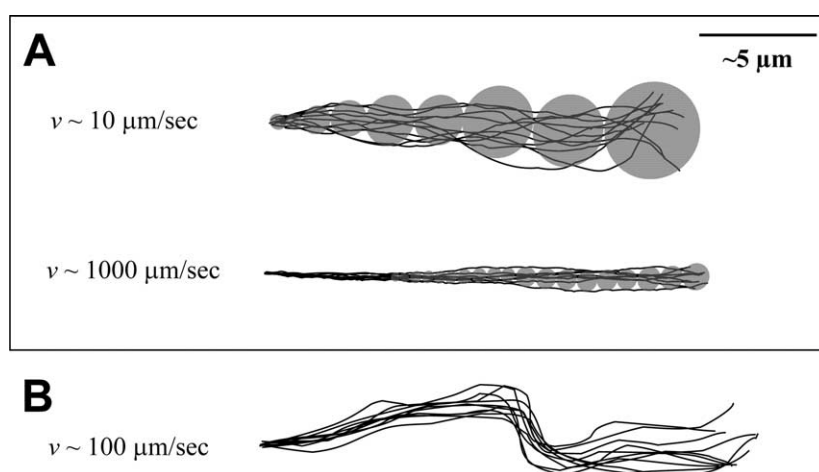

Fig. 3. Point-attached worm micelles under flow. (A) Trumpet envelopes of conformations exhibited by non-crosslinked worms. The mean flow velocity is indicated; higher velocities narrow the trumpets as predicted [17]. Lack of worm fragmentation under all such flows is consistent with strongly associated systems. (B) Configuration envelope of a crosslinked worm under flow. Note the persistent kink which resists straightening under flow.

Double bonds in the hydrophobic block of polybutadiene (PBD) allow crosslinking to be introduced by solution free radical polymerization into the worm cores [20]. Worms can thus be made even more stable and solid, emulating a classic covalent polymer chain but at a more mesoscopic scale. Such crosslinked worms often appear kinked (Fig. 3(B)). This occurs simply because the polymerization within the core is done while the worm is flexing in solution. When stuck at a point and examined under flow, such fully crosslinked worms generally just wobble and pivot about the attachment, with any kink remaining locked in and nearly unperturbed by the flow-imposed stresses.

The two types of worms, fluid or crosslinked, are two extremes at either end of a continuous stiffness scale that can be experimentally realized by blending saturated polyethylethylene (PEE) copolymer with the crosslinkable PBD copolymer. The two copolymers have already been shown with membranes to be fully miscible, and the PBD can be successfully reacted to give a range of stabilities and stiffnesses [21]. For both worms and membranes made with similar *MW* copolymers, there appears an interesting percolation of the rigidity at relatively low mole fractions of PBD (∼20%). For worms, this represents a quasi-one-dimensional fluid-to-solid transition with potentially interesting dynamics of worms blended at or near the transition point.

4. Polymersomes: viscous effects versus *MW*

Block copolymer vesicles have been made with a range of copolymers all with weight fractions, f, in the necessary range of ∼25–42% but with *MW* ranging from 3.6–20 kg/mol (Table 1). Lipids, by comparison are invariably less than 1 kDa in *MW*. Fig. 4 provides a schematic indication of the increased membrane thickness as determined by cryo-TEM. Intuition alone, in agreement with theory [22] would suggest that the resistance to membrane bending should increase with thickness, d; but the precise dependence could well reflect chain interactions within the membranes. Fig. 4 attempts to represent one key additional aspect of the physics, namely entanglements that are increasingly introduced with higher *MW*. Evidence for this has accumulated from a number of studies summarized in Table 1 and elaborated below.

One set of initial studies, which yields the most concrete numerical comparisons has made use of the method of fluorescent recovery after photobleaching [23]. The technique entails labeling a small percentage of copolymer in the membrane followed by irreversible bleaching of the fluorophore in a small region, L, of membrane using the laser in a confocal microscope. Subsequent imaging before and after a characteristic time, t, showed diffusion back into this region and provided measures of the diffusivity, $D \sim L^2/t$. Compared to measurements on lipid membranes as well as other bulk copolymers with *MW* less than the approximate entanglement molecular weight $M_e \sim 5$–10 kDa, the lower molecular weight polymersome membranes appeared to exhibit copolymer diffusivities consistent with the Rouse model, i.e., $D \sim 1/MW$. However, increasing the copolymer *MW* into this nominally entangled range but only 2.7-fold relative to the Rouse copolymers, leads to a dramatic decrease of about 50-fold in D. The one other relevant study of diffusion parallel to an ordered copolymer interface describes the diffusion of long chains as activated reptation [24]. This refers to the idea that a sufficiently long polymer must back its way out of its entanglements in the membrane and then re-insert itself in order to translate laterally. Heuristic arguments [24] suggest $D \sim (1/MW^a)\exp(1/MW)$ where a is typical of bulk reptation exponents, i.e., $a \sim 2$–3. The fact that natural cell membranes,
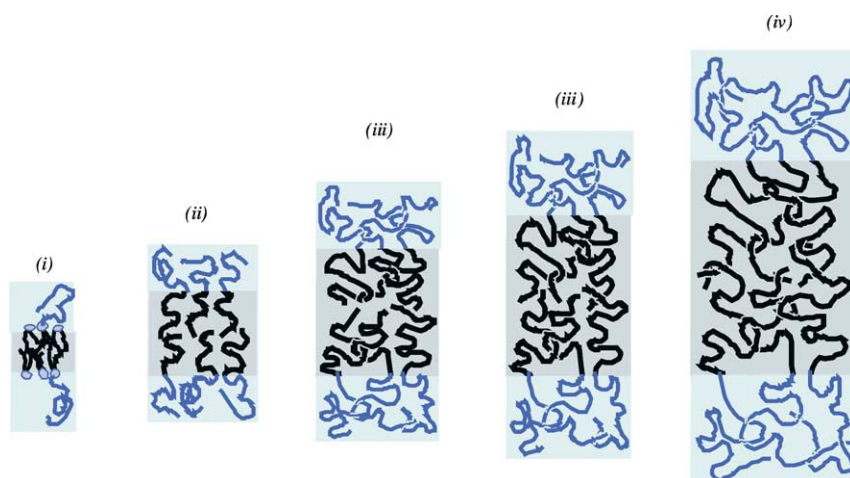
Fig. 4. Schematic of a polymersome membrane series (plus lipid) made from increasing molecular weight copolymers. The hydrophobic core thickness is d.

Table 1
Structural characteristics and hydrodynamic features of block copolymer and one representative lipid membrane. N.D. denotes not determined

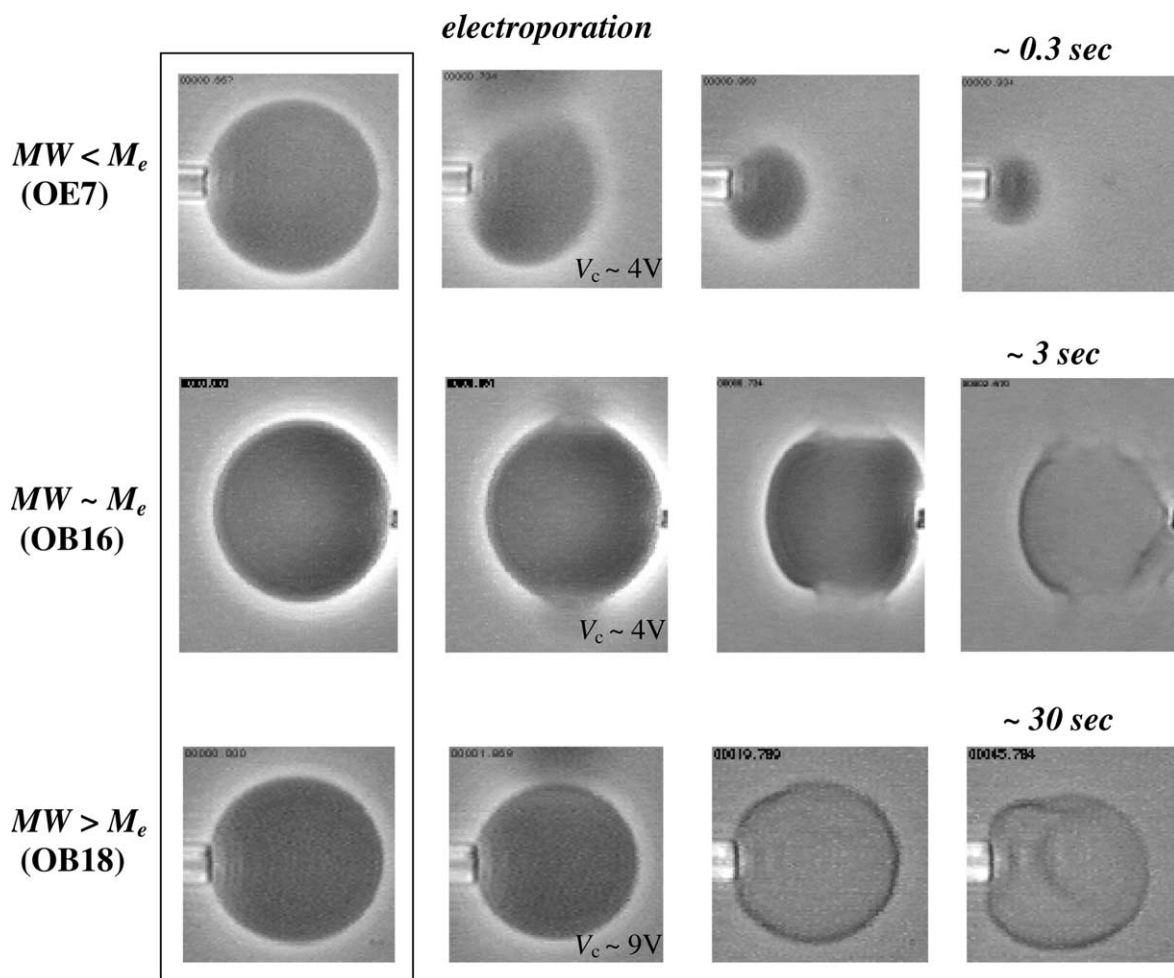|	Lipid (SOPC)	OE7, OB2	OB16	OB18	OB19
Polymer formula		$EO_{40}-EE_{37}$, $EO_{26}-BD_{46}$	$EO_{50}-BD_{55}$	$EO_{80}-BD_{125}$	$EO_{150}-BD_{250}$
MW (kg/mol)	0.79	3.9, 3.6	5.2	10.4	20.0
d (nm)	3–4	8–9	11	15	21
D ($T = 25\,°C$) (μm^2/sec)	3.8	0.12	N.D.	0.0024	N.D.
Hysteretic strain threshold ($\Delta A/A$)	none	none	none	10%	4%
Post-poration relaxation (sec)	<0.3 sec	<0.3 sec	3 sec	30–60 sec	N.D.

Fig. 5. Post-poration dynamics are protracted for polymersomes made from increasing molecular weight copolymers. M_e is the approximate entanglement molecular weight. A vesicle is pulled into a micropipette with sufficient pressure to spherize the vesicle but low enough pressures to keep the membrane tensions well below rupture. A voltage pulse is then applied in order to rupture or electroporate the membrane, and the subsequent collapse dynamics is followed.

especially with cholesterol, are so much more fluid than the polymersome membranes probably provides some additional insight into the biological benefits of membrane fluidity.

Micropipette aspiration has been extensively used to characterize polymersome membranes, following methods originally developed for blood cells [22]. While aspiration of vesicles shows the dilational elasticity of polymersome membranes is

independent of copolymer *MW* – consistent with chemistry dictating γ – evidence of hysteresis is increasingly evident for higher *MW* copolymers [11]. Table 1 lists the strain at which the release or return phase of aspiration intersects the initial aspiration phase; the smaller the strain, the bigger the hysteresis. This trend again suggests, of course, that chains take some time to disentangle and relax their convoluted interactions when the higher *MW* membranes are stressed.

One adaptation of the standard aspiration approach involves simultaneous coupling of mechanical tensioning to voltage pulses aimed at rupturing or electroporating the membrane [25]. The method clearly shows that membrane stability can increase considerably with amphiphile *MW*. Indeed, polymersome membranes are able to withstand pulses up to at least $V_c \sim 9$ V whereas lipid membranes invariably rupture at ~ 1 V or less [13]. Increasing the mechanical tension, T, while simultaneously imposing an electrical pulse, reduces the rupture voltage in a parabolic fashion with the form $T + \frac{1}{2}CV_c^2 = constant$, where *constant* generally increases with d but is $\leqslant \gamma \sim 25$–30 mN/m [11]. Classic interfacial thermodynamics provides a basis for the $T - V_c$ coupling [26], and thus implies that initial poration is relatively uninfluenced by kinetic effects such as entanglements. The post-poration process is different, however. With a small tension on the membrane, pores tend to grow, as reported for lipid vesicles in water-glycerol mixtures [27]. The results with polymersomes also suggest a viscous dependence. The higher *MW* membranes are clearly much slower in their post-poration dynamics and show slower pore growth and leakage indicating a large membrane viscosity (Fig. 5). In the specific case of OB16, we are able to measure the pore growth and existing theories fit well with the measurements [27]. Higher molecular weight polymers (OB18 and OB19) exhibit, in contrast, interior leakage (Fig. 5) for long periods of time (tens of seconds). Both results are indicative of the large membrane viscosity. These analyses will be presented elsewhere [28] where we also show that vesicles appear to largely disintegrate but, remarkably, the remnants reassemble into smaller spherical vesicles.

The one other study of polymersome viscosity effects [29] also showed highly viscous membranes and suggested that the midplane between the two leaflets of the bilayer is a source of considerable friction. Whether such results translate to the previous copolymers here is not yet clear, and requires further direct comparison of polymer systems.

5. Summary

Cell mimetic membranes and rods or worms have been made with a range of amphiphilic block copolymers having a suitably chosen f. While these and related polymersome systems are being investigated for applications such as drug delivery where processes such as permeabilization for release become crucial to understand, a more fundamental challenge lies in engineering these and the worm systems to be more active and coupled. What is needed – particularly in terms of energetic inputs – in order to make a living worm with biased end growth? Encapsulation of worms or other solutes and attachment of biomolecules would appear much less of a challenge [30] than designing new physically reasonable ways of achieving synthetic mimics of active cells.

Acknowledgements

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