Complex vesicle-based structures

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Abstract

Making nanotechnology practical is a challenge for modern research. Self-assembly is clearly going to be a necessary tool to realize practical and economic nanoscale structures. A biomimetic approach to the self-assembly of nanostructures will probably involve bilayer membrane vesicles as either the nanostructures themselves, or as templates or building blocks for more complex structures. Whether the vesicles are composed of surfactants, lipids or polymers, their stability in various environments must be optimized to suit the particular task at hand. Stabilizing the vesicles by polymerizing the surfactants themselves, or monomers templated within the vesicle or bilayer, has had a new surge of interest. Plating the vesicles, either by colloids or by polyelectrolytes, or templating the growth of various inorganic phases, has also shown promise, both in stabilizing the vesicle structure and in creating novel structures. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Vesicle; Self-assembly; Bilayer; Polymersomes; Copolymers; Polyelectrolytes; Templates; Liposomes

1. Introduction

Lipids, surfactants, polymers, and proteins self-assembled in aqueous solutions have formed the basis of a new field of ‘soft materials’ science or ‘complex fluids’ [1*]. One of the obvious practical applications of this new field is in drug-delivery systems, biosensors and other aspects of bilayer-based medicine [2]. In addition, self-assembled bilayer surfactant and lipid structures are being rediscovered as templates for the creation of new nanostructures via interactions with colloids [3*,4], assembly via ligand–receptor interactions [5,6*,7] or aggregation via polymers [8], or by coating with polyelectrolytes [9**]. New methods and materials have been used to create vesicle-like structures from copolymers [10**,11*], alternating layers of anionic and cationic polyelectrolytes [9**,12,13**] and mesoporous silica [14]. Polymerization of the surfactants within the bilayer has had a long history [15*]; the more recent approach is to use the vesicle membrane as a template for organization of the monomer prior to polymerization [15*]. In addition to templating other structures, polymerization has been used to enhance the stability of vesicles against both chemical and mechanical degradation [15*]. Other recent methods of enhancing vesicle stability, especially against attack by digestive enzymes, include the use of novel fluorinated lipids [16,17], non-ionic sugar lipids [18], or bolaform and other lipids from archaeobacterial membranes [19]. Mechanical [20,21,22**] and thermal treatment [23,24**] of lipid vesicles has also been used to create novel structures and to help understand the fundamental properties of bilayer membranes.

2. Vesicles and liposomes as ‘materials’

Encapsulating drugs in vesicles has been explored over the past 20 years, with varied levels of success
Vesicles, also known as liposomes, are spherical, closed bilayer shells, typically composed of phospholipids, cholesterol, and/or other surfactants that entrap a solvent core and separate that core from the surrounding solvent. Bangham and co-workers [26] were probably the first to recognize the potential that these lipid shells had for sequestering soluble materials for extended periods. Biomimetic vesicles are typically made from lamellar liquid crystalline dispersions of lipids, such as phosphatidylcholines, phosphatidylglycerols, cholesterol, etc., by various mechanical and/or chemical methods that act to disrupt the regular smectic stacking of the bilayers to produce separated bilayer sheets [27]. Shearing bulk-surfactant lamellar phases can also produce multilamellar liposomes that can also be used to entrap soluble materials [20,21,22].

However, any of these processes that depend on specific mechanical or chemical steps produce a microstructure that is inherently metastable. Like many other colloidal solutions, vesicles and other lipid aggregates eventually revert back to equilibrium, generally a lamellar liquid-crystal dispersion, by aggregation, fusion, etc. In the process, the vesicles can release their contents and the structure is disrupted. Equilibrium vesicles have been found, and although these vesicles form spontaneously, they tend not to be able to encapsulate an interior aqueous phase for a sufficient time to be practical [28,29], although the stability is enhanced via polymerization of monomers, such as styrene, trapped in the membrane [30].

Of course, most applications of vesicles make use of the permeability barrier imposed by the bilayer that effectively divides the solution into a distinct inside and outside. Successful recent attempts to stabilize vesicles against breakdown due to aggregation have made use of the traditional colloidal technique of steric stabilization. Lipids with large sugar headgroups (gangliosides) [31] or lipid-associated polymers, such as poly(ethylene glycol) covalently attached to phosphatidylethanolamine (PEG-lipids) [32] are incorporated into the vesicle bilayer to provide a sterically barrier to aggregation and fusion. These steric barriers prevent the bilayers from coming into contact; not surprisingly, sterically-stabilized vesicles also show an extended circulation time in the bloodstream [32].

However, there is quite a difference between adding a ‘polymer brush’ layer to a solid surface and to a relatively fluid and unstable vesicle bilayer; the phase behavior of lipids is very sensitive to small perturbations in lipid packing and inter- and intra-layer interactions. For example, mixtures of the same ganglioside lipids used to stabilize vesicles can form equilibrium, spontaneous vesicle phases [33], as do mixtures of cationic and anionic detergents [28] and lipids with alcohol co-surfactants [34]. Adding PEG-lipids or ganglioside lipids to non-bilayer forming lipids, such as unsaturated phosphatidylethanolamine, can lead to stable, bilayer vesicles that can be disrupted by removing the PEG-lipids [35]. Ions, especially polyvalent DNA [36] or F-actin [37], can interact with lipids to create completely new structures, such as cylinders and intercalated sheets. Adding PEG-lipids to more concentrated lipid–alcohol bilayers in water produces new gel phases stabilized by liquid crystalline defects [38,39].

Given these caveats, using vesicles as templates is an attractive approach to creating thin-walled nanocapsules for a variety of applications. A variety of polymerizable amphiphiles based on common surfactants and lipids that could self-assemble into vesicles in the monomer form have been synthesized. This early work proved rather disappointing, in that the extent of polymerization was often small, leading more to oligomers than high molecular-weight polymers [40,41,42]. When polymerization did occur, complicated phase separation could occur within the vesicle membrane that could even destabilize the vesicle structure. The best-documented case of this destabilization is the so-called ‘parachute’ architecture [43]. Jung et al. [43] showed that polymerizing styrene in dioctadecyldimethylammonium (DODAB) bromide vesicles led to a complete phase separation between the DODAB bilayer and a polystyrene bead. Other groups [15,30] using different surfactants or different monomers do not report this kind of phase separation. Cross-linking via a multifunctional monomer either added to the vesicle membrane or, within the surfactants themselves, seemed to minimize the phase separation and lead to hollow polymer shells [15,42,44]. However, in almost all of these schemes, the polymerization distorts the vesicle shape to some extent, either during the reaction or during processing to remove the template [15,42,44].

To avoid some of the difficulties in polymerization after vesicle formation, copolymers can be used to self-assemble into vesicle forms. Discher et al. [10] have shown that amphiphilic diblock copolymers of modest molecular weight (approx. 4000 Da) can assemble into bilayer structures in water. The polyethyleneoxide–poly(ethylene glycol) copolymer (EO40–EE37) spontaneously assembles into a variety of structures similar to surfactants — spherical and rod micelles and bilayer vesicles. The elastic properties of these bilayers were remarkably similar to lipid and surfactant bilayers, even though the bilayer membranes were approximately 8 nm thick, approximately twice as thick as typical lipid bilayers. These vesicles
could also entrap an aqueous soluble dye for extended periods of time, with a permeability coefficient somewhat lower than typical for lipid bilayers (Fig. 1).

Ihlan et al. [11] have demonstrated a somewhat more complex system that forms bilayer-like structures in chloroform. These authors used the hydrogen bonding properties of diaminopyridine- and thyamine-sidechain polymers on a polystyrene backbone. When these two polymers were mixed in chloroform solution, hydrogen bonding led to the formation of multi-μm diameter quasi-spherical aggregates that appear to have a vesicle-type hollow morphology.

### 3. Vesicles as true templates

A novel route for fabricating hollow shell structures consists of the stepwise adsorption of polyelectrolytes of opposite electric charge onto the surface of colloidal particles. This work is based on the formation of alternating thin layers of polyelectrolytes onto charged solid surfaces [45]. The process is a straightforward application of the charge reversal that often accompanies polyelectrolyte adsorption from solution [45]. An excess of polyelectrolyte is added to a charged colloidal template; these templates have included polystyrene lattices [12], melamine formamide particles [9,13,46], and glutaraldehyde-fixed red blood cells [13,47] (see Fig. 2). The latter two templates can be removed by chemical treatments without disrupting the adsorbed layer. The excess polyelectrolyte is removed by membrane filtration [9], the templates are washed with water, and the polyelectrolyte of opposite charge is added and allowed to adsorb. The process is repeated until the desired number of coating layers is reached [9].

In addition to making hollow polymer shells of polyelectrolyte, these authors have also made composite membrane shells from silica nanoparticles alternating with charged polymers [12]. They also used charged vesicles and lipids in methanol to coat the polymer shells with a lipid bilayer to enhance biocompatibility [13]. The permeation properties of these polymer shells is quite interesting — the polyelectrolyte acts like a molecular filter; low molecular-weight, uncharged molecules pass readily through the shells, while higher molecular-weight polymers or biological macromolecules do not. Adding the lipid bilayer by vesicle adsorption decreases the low molecular-weight
permeability, similar to that of conventional vesicles [13••].

Another example of charged vesicles templating charged colloidal particles showed the curious result that opposite charges can repel each other [3••,4]. Bilayer vesicles were prepared from a mixture of cationic and neutral surfactants. The vesicles were then allowed to adhere to either negatively charged substrates or colloidal particles. Despite the high charge on the vesicles, the colloidal particles separated into adhesive and non-adhesive zones. The adhesion would saturate at some optimal coverage. This counter-intuitive finding was explained by the finite concentration of counter-ions contained within the vesicle membrane assumed to be trapped and the mobility of the charged surfactant within the membrane [3••,4]. In the adhesion zone between the vesicle and the colloid, the cationic surfactant migrates to match the negatively charged surface of the colloid. This frees the trapped counter-ions within the vesicle, which then have a tendency to migrate outside the adhesion zone. This can lead to an area of charge reversal, making the vesicle membrane locally anionic, which then leads to repulsion of additional charged colloids, thereby limiting the size of the adhesion zone (Fig. 3). Further interactions between the adsorbed colloidal particles can lead to ordering of the attached colloidal particles [4].

4. Ligand–receptor interactions

Other complex structures can be formed by combining ligand–receptor interactions with lipid self-assembly [5,6•]. The ligand–receptor interaction has been used to attach vesicles to specific recognition molecules attached to cells [48], or to cross-link vesicles by the addition of a multi-site receptor, such as streptavidin [5,6•]. The linkage is typically made by using biotin covalently attached to the lipid headgroup of phosphatidylethanolamine, either directly [6•], or via an extended poly(ethylene glycol) linkage [49,50]. Multiple levels of ligand–receptor interactions can be used to encapsulate aggregates of vesicles within other vesicles to better mimic natural cell organization (Fig. 4) [5]. One drawback to using the biotin–streptavidin system is that streptavidin is a bacterial protein and can cause an immune response. By controlling the stoichiometry of the ligand–receptor interaction, the vesicles can be made to form small aggregates of 5–10 vesicles, up to super-aggregates that effectively cross-link all the vesicles in solution [6•]. Unlike other forms of colloidal aggregation that produce fractal objects of very low density, aggregating vesicles by ligand–receptor interactions produces compact, spheroidal aggregates. This is likely due to the high mobility of the lipid–ligand within the bilayer membrane [6•].

5. Conclusions

Nanoscale assembly is the current rage within the scientific and engineering community. Self-assembled nanostructures are likely to be the only possible way of producing such objects economically and practically. The self-assembly of lipids and detergents into bilayer membrane vesicles provides a template with many useful length scales — the 3–5-nm thickness of the bilayer and the 100–10 000-nm diameter of the
vesicle. Polymerization, plating with polyelectrolytes, fashioning vesicles from polymers, and assembling the vesicles into more complex structures shows the versatility of this structural form. Hopefully, this new enthusiasm for novel applications of vesicles will take advantage of the materials science community’s new appreciation of lipids as materials and a fundamental understanding of the relationships between lipids, proteins, and polymers in aqueous media will result.

Acknowledgements

The authors gratefully acknowledge support by grants from the National Science Foundation (CTS-9814399) and the MSERC program of the NSF (DMR-96-32716), the University of California STAR Biotechnology Program (S97-10), and Alliance Pharmaceuticals. D. Weitz, E. Donath and D. Discher are acknowledged for sharing their vesicle pictures. Continuing thanks are due to my current and former students and post-docs who have worked on these projects: Dr Scott Walker, Dr Michael Kennedy, Dr Hee-Tae Jung, Mr Bret Coldren and Ms Cecile Boyer.

References and recommended reading

- of special interest
- of outstanding interest


This paper presents an up-to-date perspective on the physics and chemistry of complex fluids and applications toward enhanced drug delivery.


The authors present the curious result of colloids of opposite charge repelling, rather than attracting each other.


This paper describes how to create aggregates of vesicles of various sizes by ligand–receptor interactions.


This paper reviews efforts on creating polyelectrolyte layered capsules templated on a variety of colloidal objects.


[This is the first successful effort to construct equilibrium vesicle structures from amphiphilic copolymers].


These authors have synthesized complementary side-chain polymers that aggregate into bilayer structures in organic solvents.


In addition to the polyelectrolyte shells described in [11], the authors show that bilayer membranes can be deposited on the aggregates by adsorbing charged vesicles.


This is a nice, short review of efforts to use vesicles as vehicles for...
polymerizations within the aqueous core, within the membrane, etc.


This paper shows the mechanisms by which bulk lamellar phases can be converted to multilayer ‘onions’ by shear.


This paper, and others by this group, has shown the variety of forms possible by vesicles and bilayers undergoing thermal shock via laser radiation.


This is the seminal paper on the use of lipid bilayers as diffusion barriers.


This paper gives the biomedicai perspective of vesicle-based drug delivery schemes, as well as a good introduction to the problems associated with understanding pharmacokinetics.


This is an important paper on the complex interactions of multivalent biological ions on the morphology and phase behavior of lipids.


This paper gives a nice summary of what is known about polymerization within vesicles and other self-assembled surfactant structures.


This paper clearly shows the phase separation possible between the bilayer membrane and a growing polymer bead during vesicle polymerization.


This paper gives a nice introduction and overview to the area of sequential layering of polyelectrolytes on charged surfaces.


