Adhesive and Anti-Adhesive Agents in Giant Vesicles**

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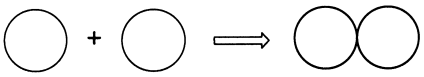
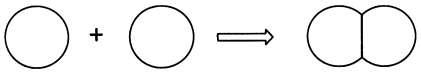
Life, it might be said, is a sticky business. Substrate and enzyme; antigen and antibody; tRNA and mRNA; leukocyte and endothelial cell; bacteriophage and bacterium; sperm and egg—all such pairs stick to each other. Sometimes, as with plaque material and arterial walls, stickiness threatens life. Other times, as with a metastasizing cancer cell, it is the absence of a normal stickiness that threatens life. The present paper is devoted to the adhesion between biomembranes comprising giant vesicles. Our goal is to identify agents and mechanisms that promote or impede adhesion between such membranes.[1]

Although excellent methods exist for studying adhesion between membrane surfaces,[2] giant vesicles (hollow bilayer spheres 10–100 μm in diameter) offer certain advantages.[3, 4] First, one can construct surfaces with a known composition and with realistic, cell-like curvatures. Second, and more importantly, one can see adhesion under a light microscope. When two giant vesicles do not adhere, they remain undeformed upon contact [Eq. (1), where the circles represent



(1)

cross-sections of the lipid spheres]. When two vesicles strongly adhere, they fuse to create a common boundary [Eq. (2)]. As will be seen, this adhesion can be temporary or permanent, depending upon the bilayer composition and the presence or absence of an agent. Natural and synthetic polymers, in single or multilayers, can also effect adhesive properties.



(2)

Single giant vesicles (believed to be unilamellar)[5, 6] were prepared by electroformation,[7] manipulated with micropipettes,[8] and observed in real time by phase-contrast microscopy. When giant vesicles are subjected to mechanical,[9] thermal,[10] chemical,[11] or electrical[12] alterations, various cytomimetic[9] events take place. These include fusion, fission, birthing, budding, and endocytosis. Many of these morphological changes are difficult or impossible to observe with smaller vesicles (30–200 nm), the study of which has, to date, comprised the bulk of vesicle (liposome) research.[13, 14]

Figure 1 shows what happens when two vesicles fail to adhere after a micropipette places them into direct contact. Vesicle pairs displaying this behavior are of three types: a) Two neutral vesicles composed of 1-palmitoyl-2-oleoylphosphatidylcholine (POPC); b) two positively charged vesicles composed of POPC with 5 mol % of cationic didodecyldimethylammonium bromide (DDAB); c) two negatively charged vesicles composed of POPC with 5 mol % of anionic 1-palmitoyl-2-oleoylphosphatidylglycerol (POPG). All experiments were carried out at room temperature, at which the host lipid (POPC) exists in the fluid liquid crystalline phase within vesicles 50–100 μm in diameter.

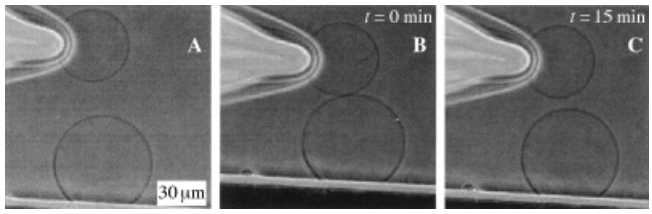


Figure 1. Phase-contrast photomicrographs of POPC vesicles, which are brought into contact with a micromanipulator. The vesicles fail to adhere and drift apart within 15 min. Immediate separation occurs when the micropipette is shifted upward.

Completely different behavior is observed when the two vesicles are of opposite charge (Figure 2). Thus, two distinct populations of vesicles were prepared by coating one Pt wire of the electroformation cell[7] with negative lipid and the other Pt wire with positive lipid. Application of a 1.0 V, 3.0 Hz alternating current produced giant vesicles on both wires, which could be brought into contact by micromanipulation. The resulting adhesion persists for at least 40 min and the unattached vesicle is free to move and explore the surface of its partner (Figure 2; a behavior that would not be observable by traditional methods, for example, light scattering).

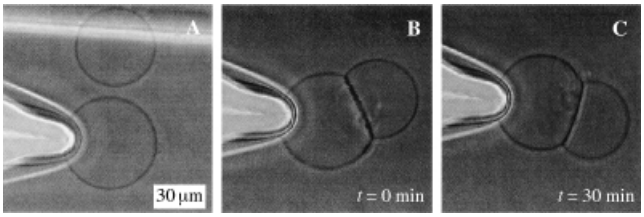


Figure 2. Adhesion between anionic vesicle composed of POPC:POPG (90:10) and a cationic vesicle composed of POPC:DDAB (90:10). Note how the POPC–DDAB vesicle moves about the surface of the POPC–POPG vesicle fixed to the pipette.

Adhesion between oppositely charged vesicles can be prevented by incorporating into both POPC vesicles 10 mole % of 1-palmitoyl-2-oleoylphosphatidylethanolamine, in which the nitrogen was bonded to a 2000 molecular weight polyethylene glycol (PEG). Later experiments showed that this level of polyethylene glycol incorporation in only one of the two vesicles also suffices to prevent adhesion. On the other hand, a 2 mole % polymer-bearing lipid does not

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prevent adhesion. These observations are reminiscent of the enhanced lifetimes of PEG-protected submicroscopic vesicles in biological fluids.[15]

An interesting time-dependent adhesion occurs when 20 mole % of 1,2-dioleoyl-phosphatidylethanolamine (DOPE) is included in the POPC bilayer. DOPE was of interest to us because of its tendency to form an inverted hexagonal (H_{II}) phase, which can facilitate membrane fusion.[16, 17] Adhesion between two oppositely charged vesicle occurs as usual (Figure 3). However, after 10–15 min, the vesicles are seen to lose their adhesion and drift apart. If the DOPE level is reduced to 10 mole %, then the vesicles drift apart only after 25 min.

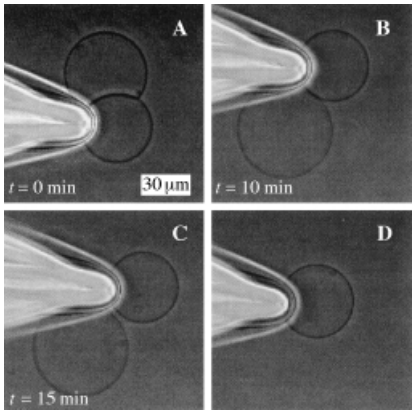
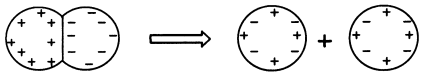


Figure 3. Two oppositely charged vesicles composed of POPC:POPG:-DOPE (75:5:20) and POPC:DDAB:DOPE (75:5:20) showing A) adhesion at 0 min; B) reduced adhesion after 10 min; C) complete separation after 15 min; D) diffusion of separated vesicle from field of view.

The simplest explanation is that the DOPE allows the exchange of charged lipids across the common boundary. Once the charge in each membrane becomes neutralized, the vesicles are free to separate [Eq. (3)]. In 1991, Wimley and Thompson[18] demonstrated that a phosphatidylethanolamine enhances phospholipid exchange among 110 nm vesicles, but Figure 3 is the first visual evidence for such an effect.



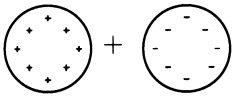
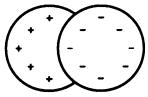
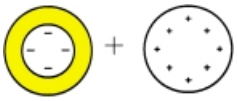
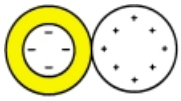
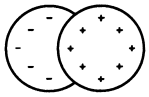
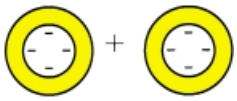
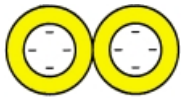
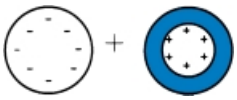
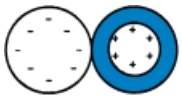
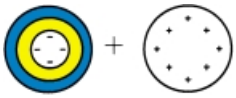
(3)

Poly-L-lysine, a polycation known to induce morphological changes or rupture in both model and cell-membrane systems,[19, 20] does not promote adhesion between two neutral POPC vesicles. However, when poly-L-lysine (0.2 μM, M_r = 244 kD) is added to two POPC vesicles rendered anionic with 10 mole % POPG, the vesicles adhere tightly. It is as if the poly-L-lysine acts as an electrostatic glue. One wonders if an imposed selective adhesion might provide a mechanism for confining cancer cells to their primary tumor site.

The preceding served as a prelude to more sophisticated experiments involving two biomedically important macromolecules: polyvinylpyrrolidone (PVP, a nonionic polymer)[21] and heparin (an anionic polysaccharide).[22] PVP has a known affinity for anionic compounds,[23] and heparin interacts with cationic bilayers.[24]

Entry 2 (Table 1) shows how the addition of PVP (25 μM, M_r = 40 kD) to a mixed sample of anionic and cationic vesicles prevents adhesion. This effect must be caused by a PVP coating (yellow) of the anionic vesicle, because as shown in entry 3 a cationic vesicle (exposed to the PVP in a separate apparatus and then transferred by pipette to an anionic vesicle) does adhere. Two anionic vesicles, both with adsorbed PVP, do not adhere (entry 4). In entry 5, a cationic vesicle was coated with heparin (blue; 1 μM, M_r = 3 kD) prior to its introduction to an anionic vesicle. The usual anionic/cationic-vesicle adhesion (entry 1) is thereby prevented.

Table 1. Interactions of anionic and cationic vesicles coated with nonionic PVP (yellow) and anionic heparin (blue).

Entry	Type of interaction	Effect
1		
2		
3		
4		
5		
6		

A double-coating experiment is shown in entry 6: A homogeneous population of anionic vesicles is first exposed to 25 μM PVP for 10 min, followed by a 1 μM heparin exposure. Vesicle transfer to a separately prepared colony of cationic vesicles produced adhesion. Had the heparin not adsorbed onto the PVP, no adhesion would have occurred (entry 2). Clearly, PVP can effectively bind anionic heparin to anionic vesicles. And heparin can serve as a membrane adhesive (entry 6) or anti-adhesive (entry 5), depending upon the charge properties of the system.

In summary, a series of diverse experiments, in which adhesion is promoted or prevented, demonstrates the utility of giant vesicles in assessing the stickiness of organic substances.

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