Membrane Sealing by Polymers

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ABSTRACT: An intact cell membrane serves as a barrier, controlling the traffic of materials going into and out of the cell. When the integrity of the membrane is compromised, its transport barrier function is also disrupted, leaving the cell vulnerable to necrosis. It has been shown that triblock copolymer surfactants can help seal structurally damaged membranes, arresting the leakage of intracellular materials. Using model lipid monolayers along with concurrent Langmuir isotherm and fluorescence microscopy measurements as well as surface X-ray scattering techniques, the nature of the interaction between lipids and a particular family of triblock copolymers in the form poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) is examined. The polymer is found to selectively insert into membranes where the lipid packing density is below that of an intact cell membrane, thus localizing its sealing effect on damaged portions of the membrane. The inserted polymer is "squeezed out" of the lipid film when the lipid packing density is increased, suggesting a mechanism for the cell to be rid of the polymer when the membrane integrity is restored.

KEYWORDS: cell membrane; lipid bilayer; poloxamer; poloxamine; surfactant

THE CELL MEMBRANE

The cell membrane separates materials inside the cell from those in the environment. In essence, it plays the crucial role of a gatekeeper, acting as a permeable barrier for transport into and out of the cell, thus regulating the molecular and ionic content of the intracellular medium. The majority of the energy required to sustain cellular function is expended in maintaining large differences in electrolyte ion concentrations across the cell membrane. The lipid bilayer constituting the membrane provides the necessary ionic diffusion barrier that makes it energetically possible to maintain large transmembrane ion concentration gradients. The lipid bilayer serves this role remarkably well by establishing a nonpolar region through which an ion must pass to cross the membrane. However, cell membranes consist typically of 30% proteins, many of which facilitate and regulate membrane ion transport. These membrane protein effects combine to make the mammalian cell membrane roughly 10^6 times more conductive to ions than the pure lipid bilayer.[1]

The mammalian cell membrane is essentially a two-dimensional sheet-like structure with a typical thickness of 60 to 100 Å. Forces that hold the lipid and protein molecules together in this assembly are not strong covalent or ionic bonds, but rather

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Ann. N.Y. Acad. Sci. 1066: 310–320 (2005). © 2005 New York Academy of Sciences.
doi: 10.1196/annals.1363.018

the much weaker forces, such as van der Waals, hydrophobic, hydrogen bonding, and screened electrostatic interactions. The Fluid Mosaic model proposed in 1972[2] depicts the membrane lipid bilayer as a passive entity, serving no special purpose other than providing a solvent for the membrane proteins to freely diffuse within the membrane. Over the last decade, however, evidence has emerged to suggest that compositional heterogeneity in the lipid bilayer within the membrane is important for membrane trafficking, signal transduction, selective protein attachments, and biomolecular reactions. Such membrane heterogeneity has been proposed to be the result of self-organization of various lipid species into domains[3] or rafts.[4] Irrespective of where the lipids reside, they all exhibit rotational and lateral diffusion within the membrane. Occasionally, small separations in the lipid packing order occur, producing transient structural defects with lifetimes on the order of nanoseconds. This lifetime is sufficient to permit passage of small solutes including water. The lifetime and size of these transient pores are influenced by temperature, electric field strength in the membrane, and polymers absorbed onto the membrane interface. The integrity of the membrane bilayer is essential for maintaining physiological transmembrane ionic concentration gradients at an affordable metabolic energy cost.

CELL MEMBRANE DAMAGE

Despite its critical role in supporting life, the lipid bilayer is quite fragile compared to other biological macromolecular structures. Many forms of trauma can disrupt the transport barrier function of the cell membrane. Loss of cell membrane integrity occurs in tissues at supraphysiologic temperatures as in the case of thermal burns, with very intense ionizing radiation exposure, in frostbite, in barometric trauma, and with exposure to strong electrical forces in electrical shock. Electrical shock is the paradigm for necrosis primarily mediated by membrane permeabilization. Skeletal muscle and nerve tissue exposed to strong electrical fields (greater than 50 V/cm) can experience membrane damage by at least three distinct physiochemical processes—electroporation, heat-mediated membrane poration, and electroconformational membrane protein denaturation.

When the bilayer structure is damaged, ion pumps cannot keep pace with the increased diffusion of ions across the membrane. Under these circumstances, the metabolic energy of the cell is quickly exhausted, leading to biochemical arrest and necrosis. Defects formed in the membrane can be stabilized by membrane proteins anchored in the intra- or extracelluar space. Chang and Reese[5] have demonstrated that stable structural defects—"pores" in the range of 0.1 μm—occur in electroporated cell membranes. In other cases, the translateral motion of the lipids, normally restricted by anchored proteins may cause the membrane to form bubbles as a result of the expansion of electroporated cell membranes, compromising the local lipid packing and leading to an enhanced permeability.

SURFACTANT SEALING OF CELL MEMBRANES

Sealing of porated or permeabilized cell membranes is an important, naturally occurring process. Fusogenic proteins induce sealing of membranes following exocytosis by creating a low-energy pathway for the flow of phospholipids across the

defect or to induce fusion of transport vesicles to plasma membranes. Membrane sealing has also been accomplished using surfactants. The amphiphilic properties of poloxamers, a group of triblock copolymers, are able to interact with the lipid bilayer to restore its integrity. Poloxamer 188 (P188) has been used widely in medical applications since 1957, mainly as an emulsifier and anti-sludge agent in the blood.[6] Thus, most investigations on the sealing capabilities of synthetic surfactants have focused on P188 due to its already established medical safety record.

The first demonstration was that P188 could seal cells against loss of carboxyfluorescein dye after electroporation.[7] Low molecular weight (10 kDa) neutral dextran was unsuccessful in producing the same effect. In the following years, it has been shown that P188 can also seal membrane pores in skeletal muscle cells after heat shock[8] and enhance the functional recovery of lethally heat-shocked fibroblasts.[9] More recently, P188 has been shown to protect against glutamate toxicity in the rat brain[10] and protect embryonic hippocampal neurons against death due to neurotoxic-induced loss of membrane integrity[11,12] and reduce the leakage of normally membrane impermeant calcein dye from high-dose irradiated primary isolated skeletal muscle cells.[13] Other surfactants, such as poloxamine 1107 (P1107), have been shown to reduce testicular ischemia-reperfusion injury,[14] hemoglobin leakage from erythrocytes after ionizing radiation,[15,16] and propidium iodine uptake of lymphocytes after high-dose ionizing irradiation.[17] In all the aforementioned investigations, the observed phenomena were attributed to sealing of permeabilized cell membranes by the surfactants. In addition, the effect of P188 infusions in reducing duration and severity of acute painful episodes of sickle cell disease is presently also explained by beneficial surfactant-erythrocyte membrane interactions.[18]

TRIBLOCK COPOLYMER SURFACTANTS

Poloxamers and poloxamines belong to a class of water-soluble triblock copolymers often abbreviated as PEO-PPO-PEO, with PEO and PPO representing poly(ethylene oxide) and poly(propylene oxide), respectively. The PEO chains are hydrophilic due to their short carbon unit between the oxygen bridges, whereas the PPO center is hydrophobic due to the larger propylene unit (FIG. 1). Commercially available poloxamers and poloxamines have both PEO chains of similar length in a

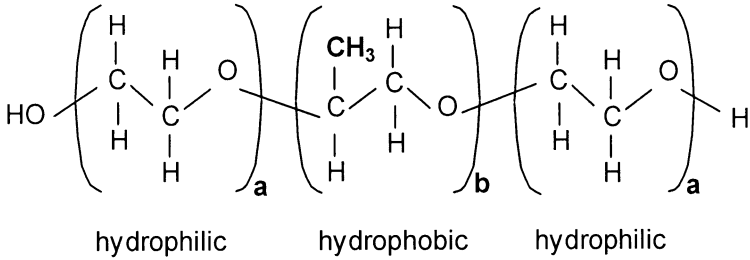

FIGURE 1. Chemical structure of poloxamers. The series of different poloxamers is constituted through varying numbers and ratios for **a** and **b**.

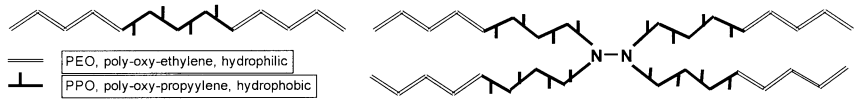

FIGURE 2. Schematic drawing to illustrate structural differences between poloxamers (*left*) and poloxamines (*right*). The PEO and PPO chain lengths vary among the members of the surfactant families.

particular copolymer. The lengths of the hydrophilic and the hydrophobic chains and their lengths ratios (FIG. 1, a vs. b) can vary tremendously, forming a large group of copolymers widely used in industrial applications as emulsifying, wetting, thickening, coating, solubilizing, stabilizing, dispersing, lubricating, and foaming agents.[21] The poloxamine series is slightly different from the poloxamer series in that the hydrophobic center consists of two tertiary amino groups each carrying two hydrophobic PPO chains of equal length each followed by a hydrophilic PEO chain. Thus, it still is a triblock copolymer but it is much bulkier than poloxamers (FIG. 2).

The poloxamer series covers a range of liquids, pastes, and solids, with molecular weights varying from 1100 to about 14,000 Da. The ethylene oxide:propylene oxide weight ratios range from about 1:9 to about 8:2. P188 has an average molecular weight of about 8400 kDa. It is prepared from a 1750-Da average molecular weight hydrophobe (29 propylene oxide units), and its hydrophile (76 ethylene oxide units) comprises about 80% of the total molecular weight. In the nomenclature of the poloxamers, the last digit (here 8) indicates the weight percentage of the hydrophilic part of a surfactant (here 80%). Thus, the poloxamers 108, 188, 238, and 288 are a series with increasing overall chain lengths but constant 80% hydrophile weight percentage. Among the group of poloxamers named P183, P185, and P188, the length of the hydrophobic chains stay constant at about 1800 Da (indicated by the first two digits, here 18) but the hydrophile weight percentage varies from 30% to 80%.

Physicochemical Properties of Triblock Copolymer Surfactants

A characteristic physicochemical parameter of surfactants is their critical micelle concentration (CMC). Above their CMC, surfactants self-aggregate to micelles causing the (active) surfactant monomer concentration to remain constant (= CMC) independent of the total surfactant concentration. Triblock copolymer surfactants, unlike conventional nonionic surfactants, do not form micelles at a critical micelle concentration. Instead, aggregation occurs over a broad concentration range, which is referred to as the aggregation concentration range. The limiting aggregation concentration (LAC) is the point at which the surfactant reaches saturation, which would correspond to the more conventional CMC (BASF Corporation 1999). This aggregation behavior of the triblock copolymers most likely accounts for the widespread values of CMCs reported in the literature, reflecting its dependence on the particular determination method used. For example, the CMC of P188 at 30° has been given as ≥100 mg/mL by Kabanov *et al.*[19] and 12.5–51.7 mg/mL by Alexandritis and Hatton.[20] In *in vitro* membrane sealing applications, P188 is typically used at concentrations well below the CMC of 0.1–1.0 mM corresponding to about 1–10 mg/mL. On the

basis of these results, the surfactant monomer is presumed to be the active agent not the surfactant micelle.

LIPID–POLOXAMER INTERACTIONS

Given the membrane-sealing capability of poloxamer, one can envision using the poloxamer as a membrane sealant for therapeutic purposes. The design of an effective therapy for membrane sealing requires a good understanding of the nature of the interaction between lipid membranes and poloxamers. Ideally, the poloxamer should be able to discriminate between damaged and healthy cell membranes, interacting only with the former and not interfering with the latter. Moreover, once its presence is no longer needed (i.e., when the membrane structural integrity is restored), an exit mechanism for the poloxamer from the previously damaged membrane should be in place so that the poloxamer would not inhibit the cell healing process. Furthermore, elucidation of the mechanism by which the poloxamer helps seal the damaged membrane should aid the design of suitable polymer or polymers for therapeutic purposes.

Poloxamer and Lipid Monolayers

Although cell membranes are made up of lipid bilayers, the monolayer system provides a good mimic of the outer leaflet, with the aqueous subphase acting as the

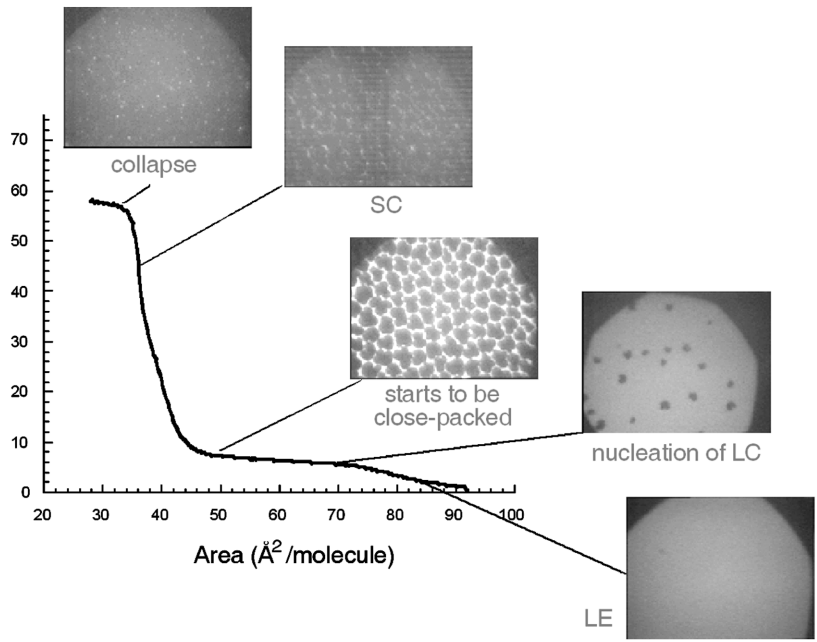

FIGURE 3. Surface pressure–area isotherm for DPPG at 30°C on pure water. The corresponding fluorescence micrographs are shown.

extracellular matrix. A Langmuir monolayer is two-dimensional (2D) film formed by a single layer of insoluble lipid molecules at the air–liquid interface. Using surface pressure–area isotherms,[22] one can observe that decreasing the lipid's surface area at the interface induces a series of 2D phase transitions.[23–29] At very high areas per lipid molecule, the molecules at the air–water interface exist in a 2D gas-like (G) state. Upon reduction of surface area by lateral compression, the monolayer condenses from the G state to an isotropic 2D fluid state known as the liquid-expanded (LE) phase. A further decrease in surface area causes a transition from the LE phase to the anisotropic condensed (C) phase. Compression beyond the minimum surface area needed for each molecule destabilizes the 2D monolayer film, resulting in the eventual collapse of the film. FIGURE 3 shows a typical surface pressure–area isotherm for dipalmitoylphosphatidylglycerol (DPPG) with the corresponding surface morphology obtained by fluorescence microscopy. The morphological images were obtained by incorporating a small amount of dye into the monolayer. Due to steric hindrance, the dye molecules preferentially partition into the disordered phase, rendering it bright and leaving the ordered phase dark.

Langmuir lipid monolayers have been extensively used as model biological membranes,[27] with the monolayer acting a good 2D model for studying interactions between different surfactants residing in the aqueous subphase and various lipids or lipid mixtures constituting the outer leaflet of the membrane surface. Langmuir troughs can be used to alter the surface area for a known amount of spread lipid accumulated at the air–water interface. The packing density of the lipid can thus be

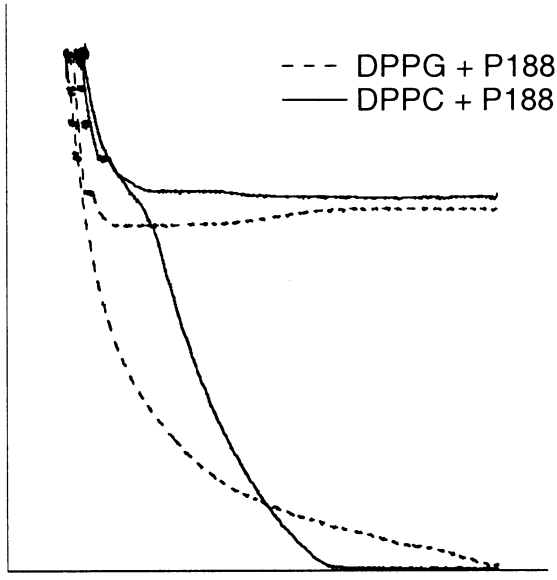

FIGURE 4. DPPC and DPPG monolayers on pure water at 30°C with P188 injected into the subphase. No change in the area was found for both cases until the surface pressure was lowered to several mN/m below the bilayer equivalent pressure.

easily controlled to simulate cell membrane damage. By measuring the extent to which these transitions are affected by the presence of poloxamers, we can gain insight into the incorporation of P188 into the monolayer.

Do Poloxamers Interact Preferentially with Damaged Membranes?

To address the question as to whether poloxamers interact preferentially with damaged membranes, we have examined the interaction of poloxamer P188 with both anionic phospholipids DPPG and zwitterionic dipalmitoylphosphatidylcholine (DPPC). An intact membrane was mimicked by compressing a spread lipid film to the bilayer equivalent surface pressure of 30 mN/m; the pressure was held constant by adjusting the surface area via a feedback mechanism. P188 was then injected into the subphase at this pressure, and the surface area of the film was monitored. Insertion of the poloxamer into the lipid film would result in an area increase while desorption of lipids into the subphase by the poloxamer would lead to a decrease in the area. For a lipid film at the bilayer equivalent pressure, no immediate change in the area per molecule (FIG. 4) or morphology was observed for a period of 10 min. Subsequently, the surface pressure was lowered to 28 mN/m, but still no observable change was detected. A pressure step-down procedure was then adopted until a low level of P188 insertion was observed at 22 mN/m. Because this change in the effective area per lipid molecule was only approximately 3 $Å^2$ for DPPG after 10 min, the surface pressure was lowered again to 20 mN/m. Rapid insertion of P188 into the DPPG monolayer was detected at this pressure with an overall change in an area per molecule of 74 $Å^2$, or until the barriers were expanded to their original position (see expansion in FIG. 4).

FIGURE 5A–C shows the morphology of a monolayer of DPPG on a water subphase at 30°C before and after P188 injection. Before injection, the condensed flower-shaped domains of DPPG occupy a much higher area fraction than the LE phase at 30 mN/m (FIG. 5A). Upon the insertion of P188 at 20 mN/m, the condensed domains become elongated, forming a more network-like structure with various-sized domains linked (FIG. 5B). In addition, there is a drastic increase in the percentage of LE or disordered phase, indicating the disordering of lipid molecules by the incorporation of P188. An additional phase of intermediate brightness is also observable (FIG. 5C).

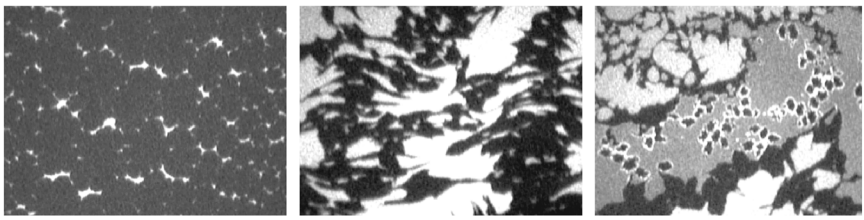

FIGURE 5. Fluorescence images showing the effect of P188 insertion into a DPPG monolayer at 30°C on pure water. P188 cannot pack well with the ordered lipid phase and preferentially associate with the disordered lipids.

Constant surface pressure injection experiment with DPPC gave similar results, with no observable change in area at the bilayer equivalent pressure after P188 administration but with substantial polymer insertion when the surface pressure was lowered to 22 mN/m (see FIG. 4). Similar morphological changes were observed upon P188 insertion.

Together these experiments suggest that P188 would only interact with compromised bilayers where the local lipid packing density is reduced and would not nonspecifically insert into membranes that were not affected. Moreover, as similar injection results were obtained for DPPC and DPPG monolayers, the insertion of the poloxamer is not influenced by the electrostatics of the lipid head group.

What is the Fate of the Poloxamer upon Cell Healing?

To determine whether there exists a mechanism for the poloxamer inserted in the damaged membrane to leave the membrane when the integrity of the once structurally compromised membrane is restored, we have examined the ability of the inserted poloxamer to retain in the model membrane at high lipid packing densities. Just as in the the injection experiments described above, the monolayer material was spread at the interface at a low surface density ($\pi \cong 0$ mN/m), but unlike in the previous case, P188 was introduced to the subphase before the lipid monolayer was compressed. The entire assembly was left undisturbed for five minutes before lateral compression commenced.

The addition of P188 to each lipid monolayer instantly displayed a drastic increase in surface pressure, from 0 mN/m to approximately 20 mN/m, close to the

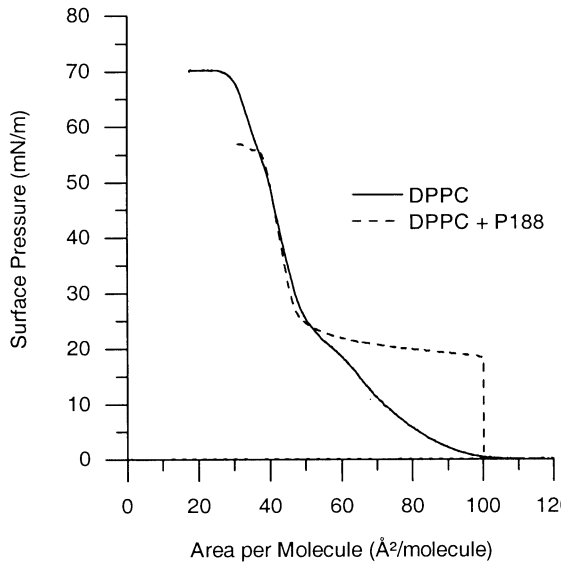

FIGURE 6. Isotherms of DPPC and P188-treated DPPC monolayers. The two isotherms overlap at surface pressures beyond 25 mN/m, indicating that the poloxamer is "squeezed-out" at high pressures.

equilibrium spreading pressure of pure P188. This high surface activity probably aids in its absorption and facilitates its insertion into lipid monolayers. The heterogeneous lipid-poloxamer system was then compressed fully. FIGURE 6 shows that as DPPC was compressed to high surface pressures, the isotherms of the poloxamer-pretreated monolayers reverted to those of the pure lipids, suggesting that P188 had been eliminated from the system. Similar results have been obtained for DPPG. These observations suggest that P188 activity is localized, capable of incorporating itself into the monolayers only when the film pressure is several mN/m below the bilayer equivalent pressure. When the lipids regain the tighter packing density found in intact cells, however, P188 cannot maintain its position within the lipid film and is "squeezed out" or eliminated as its association with the lipid layer is no longer detectable. The incapability of P188 to sustain its involvement in the system at high surface pressures can be beneficial in terms of its application. After insults of traumas such as electroporation that damage the barrier function of the cell membrane, the cell may activate a self-healing process that eventually restores the structural integrity of the bilayer. Consequently, as the cell heals and the lipid packing of the membrane is regained, P188 can be easily removed from the cell membrane.

What is the Underlying Mechanism for This Sealing Action?

A hint about the mechanism for the poloxamer-sealing action comes from experiments in which the area, instead of the surface pressure, was held constant. Here, the pure lipid monolayer was first compressed to 20 mN/m, and P188 was injected at a constant area allowing the surface pressure to increase should the polymer inserts. In the case of both DPPG and DPPC, there were dramatic surface pressure increases as a result of P188 administration (FIG. 5). Such an increase in surface pressure is indicative of tighter packing. These results therefore point to the ability of P188 to effectively insert into the damaged region of the membrane where the local lipid-packing density is reduced. By so doing, the poloxamer helps increase the local packing density.

We have recently reported that P188 changes the phase behavior and morphology of both zwitterionic DPPC and anionic DPPG monolayers.[30] P188 is found to insert into both films at surface pressures equal to and lower than ~ 22 mN/m at 30°C; this pressure corresponds to the maximal surface pressure attained by P188 on a pure water subphase. Similar results for the two phospholipids indicate that P188 insertion is not influenced by head-group electrostatics, which is not surprising as the polymer is nonionic. Because the equivalent pressure of a normal bilayer is on the order of 30 mN/m, the lack of P188 insertion above 22 mN/m further suggests that the poloxamer selectively adsorbs into damaged portions of the membrane, thereby localizing its effect. P188 is also found to be squeezed out of monolayers at high surface pressures, suggesting a mechanism for the cell to be rid of the poloxamer when the membrane is restored.[30] This squeeze-out hypothesis has also been proposed previously by Weingarten *et al.*,[31] based on their P188/PC-monolayer compression experiments.

Recent surface X-ray diffraction experiments further demonstrate that the insertion of poloxamers into a lipid film with low packing density indeed leads to a tighter packing of the lipid molecules.[33] By physically occupying part of the surface area, the adsorbed poloxamers leave the lipid molecules a smaller surface area to span and hence help tighten their packing. X-ray reflectivity results, on the other hand, show

that at high surface pressures lipid films with and without poloxamers in the subphase exhibit identical electron density profiles.[34] This signifies the absence of any poloxamer in the lipid matrix and corroborates the "squeeze-out" of poloxamers at high surface pressures (or when normal lipid packing density is restored) revealed by isotherm measurements.

Polymer Design

Do surfactant monomers interact only with disrupted parts of the membrane, sealing the pores? Do they interact with the entire bilayer, altering certain membrane properties that result in its restoration (e.g., decreased fluidity)?[35,36] Does the glycocalix play a role in the sealing process? Because there is a large variety of surfactants with different hydrophilic/hydrophobic proportions, a surfactant other than P188 might have different interactions with bilayer membranes that may be better suited to seal membranes of a specific cell or useful for a particular type of injury. In considering a transmembrane scenario for its sealing mechanism, the chain length of the hydrophobic center part, including its 3D folding, can be expected to accommodate within the thickness of the lipid bilayer. The length of the hydrophilic chain might influence the strength of the interaction between the permeabilized membrane and the surfactant and thereby influence the polymer's effectiveness as a membrane *sealant*. Poloxamines, might be more effective in restoring the membrane integrity in some instances due to their overall bulkier hydrophobic center and four hydrophilic chains, providing a stronger anchor to the membrane through increased interactions with hydrophilic lipid head groups. A thorough understanding of the structure–activity of these polymers is clearly needed to better design and develop them as sealing agents.

REFERENCES

1. SCHANNE, P.F. & E.R.P. CERETTI, 1978. Impedance Measurements in Biological Cells. Wiley. New York.
2. SINGER & NICOLSON, 1972. Fluid mosaic model of structure of cell-membranes. Science **175:** 720.
3. MOURITSEN, O.G. 1998. Biol. Skr. Dan. Vid. Selsk. **49:** 47.
4. SIMONS, K. & E. IKONEN. 1997. Nature **387:** 569.
5. CHANG, D.C. & T.S. REESE. 1990. Changes in membrane structure induced by electroporation as revealed by rapid-freezing electron microscopy. Biophys. J. **58:** 1–12,
6. SCHMOLKA, I.R. 1994. Physical basis for poloxamer interactions. Ann. N.Y. Acad. Sci. **720:** 92–97.
7. LEE, R.C., P. RIVER, F.-S. PAN, L. JI & R.L. WOLLMANN. 1992. Surfactant-induced sealing of electropermeabilized skeletal muscle membranes in vivo. Proc. Natl. Acad. Sci. USA **89:** 4524–4528.
8. PADANILAM, J.T., J.C. BISCHOF, R.C. LEE, *et al.* 1994. Effectiveness of poloxamer 188 in arresting calcein leakage from thermally damaged isolated skeletal muscle cells. Ann. N.Y. Acad. Sci. **720:** 111–123.
9. MERCHANT, F.A., W.H. HOLMES, M. CAPELLI-SCHELLPFEFFER, *et al.* 1998. Poloxamer 188 enhances functional recovery of lethally heat-shocked fibroblasts. J. Surg. Res. **74:** 131–140.
10. FRIM, D.M., D.A. WRIGHT, D.J. CURRY, *et al*. 2004. The surfactant poloxamer-188 protects against glutamate toxicity in the rat brain. NeuroReport **15:** 171–174.
11. MARKS, J.D., W. CROMIE & R.C. LEE. 1998. Nonionic surfactant prevents NMDA-induced death in cultured hippocampal neurons. Soc. Neurosci. Abs. **24:** 462.

12. MARKS, J.D., C-Y PAN, T. BUSHELL, *et al.* 2001. Amphiphilic tri-block copolymers provide potent, membrane-targeted neuroprotection, FASEB J. doi:10.1096/fj.00-0547fje, 2001.
13. HANNIG, J. & R.C. LEE. 2000. Structural changes in cell membranes after ionizing electromagnetic field exposure. IEEE Trans. Plasma Sci. **28:** 97–101.
14. PALMER, J.S., W.J. CROMIE & R.C. LEE. 1998. Surfactant administration reduces testicular ischemia-reperfusion injury. J. Urol. **159:** 2136–2139.
15. HANNIG, J., D.J. CANADAY, M. BECKETT, *et al.* 2000. Sealing of membranes permeabilized by ionizing radiation. Radiat. Res. **154:** 171–177.
16. GREENEBAUM. B., K. BLOSSFIELD, J. HANNIG, *et al.* 2004. Poloxamer 188 prevents acute necrosis of adult skeletal muscle cells following high-dose irradiation, Burns **30:** 539–547.
17. TERRY, M.A., J. HANNIG, C.S. CARRILLO, *et al.* 1999. Oxidative cell membrane alteration: evidence for surfactant mediated sealing. Ann. N.Y. Acad. Sci, **888**: 274–284.
18. ADAMS-GRAVES, P., A. KEDAR, M. KOSHY, *et al.* 1997. RheothRx (Poloxamer 188) injection for the acute painful episode of sickle cell disease: a pilot study. Blood **90:** 2041–2046.
19. KABANOV, A.V., I.R. NAZAROVA, I.V. ASTAFIEVA, *et al.* 1995. Micelle formation and solubilization of fluorescent probes in poly(oxyethylene-β-oxypropylene) solutions. Macromolecules **28:** 2303–2314.
20. ALEXANDRIDIS, P. & T.A. HATTON. 1995. Poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) block copolymer surfactants in aqueous solutions and at interfaces: thermodynamics, structure, dynamics, and modeling. Colloids Surf. **96:** 1–46. 1995.
21. CHU, B. & Z. ZHOU, 1996. Physical chemistry of polyoxyalkylene block copolymer surfactants. Surf. Sci. Ser. **60:** 67–144.
22. GAINES, G.L. 1966. Insoluble Monolayers at Liquid-Gas Interface. Interscience. New York.
23. ANDELMAN, D., F. BROCHARD, C. KNOBLER & F. RONDELEZ. 1994. Structures and phase transitions in Langmuir monolayers. *In* Micelles, Membranes, Microemulsions and Monolayers. W. Gelbart, A. Ben-Shaul & D. Roux, Eds.: 559–602. Springer-Verlag. New York.
24. KAGANER, V.M., H. MÖHWALD & P. DUTTA. 1999. Structure and phase transitions in Langmuir monolayers. Rev. Modern Phys. **71:** 779–819,
25. KNOBLER, C.M. & R.C. DESAI. 1992. Phase-transitions in monolayers. Annu. Rev. Phys. Chem. **43:**207–236.
26. MCCONNELL, H.M, 1991. Structures and transitions in lipid monolayers at the air-water interface. Annu. Rev. Phys. Chem. **42:** 171–195.
27. MÖHWALD, H, 1990. Annu. Rev. Phys. Chem. **41:** 441–476.
28. MÖHWALD, H, 1993. Surfactant layers at water surfaces. Rep. Prog. Phys. **56(5):** 653–685.
29. WEIS, R.M, 1991. Fluorescence microscopy of phospholipid monolayer phase-transitions. Chem. Phys. Lipids **57:** 227–239.
30. MASKARINEC, S.A., J. HANNIG, R.C. LEE & K.Y.C. LEE. 2002. Direct observation of Poloxamer 188 insertion into lipid monolayers. Biophys. J. **82:** 1453–1459.
31. MASKARINEC, S.A. & K.Y.C. LEE. 2003. Comparative study of poloxamer insertion into lipid monolayers. Langmuir **19:** 1809–1815.
32. WEINGARTEN, C., N.S.S. MAGALHAES, A. BASZKIN, *et al.* 1991. Interaction of non-ionic APA copolymer surfactant with phospholipid monolayers. Int. J. Pharmacol. **75:** 171–179.
33. WU, G., C. EGE, J. MAJEWSKI, *et al.* 2004. Lipid corralling and poloxamer squeeze-out in membranes. Phys. Rev. Lett. **93:** 02810.
34. WU, G, J. MAJEWSKI, C. EGE, *et al.* 2005. Interaction between lipid monolayers and Poloxamer 188: an X-ray reflectivity and diffraction study. Biophys. J. **89:** 3159–3173.
35. SHARMA, S, K. STEBE. K. MURPHY & L. TUNG. 1996. Poloxamer 188 decreases the susceptibility of artificial lipid membranes to electroporation. Biophys. J. **71:** 3229–3241.
36. BAEKMARK, T.R., S. PEDERSEN, K. JØRGENSEN & O.G. MOURITSEN, 1997. The effects of ethylene oxide containing lipopolymers and tri-block copolymers on lipid bilayers of dipalmitoylphosphatidylcholine. Biophys. J. **73:** 1479–1491.