BRIEF COMMUNICATION

ANALYSIS OF ADHESION OF LARGE VESICLES TO SURFACES

EVAN A. EVANS, *Department of Biomedical Engineering, Duke University, Durham, North Carolina 27706 U.S.A.*

ABSTRACT An experimental procedure that can be used to measure the interfacial free energy density for the adhesion of membranes of large vesicles to other surfaces is outlined and analyzed. The approach can be used for both large phospholipid bilayer vesicles and red blood cells when the membrane force resultants are dominated by isotropic tension. The large vesicle or red cell is aspirated by a micropipet with sufficient suction pressure to form a spherical segment outside the pipet. The vesicle is then brought into close proximity of the surface to be tested and, the suction pressure reduced to permit adhesion, and the new equilibrium configuration is established. The mechanical analysis of the equilibrium shape provides the interfacial free energy density for the surface affinity. With this approach, the measurable range of membrane surface affinity is 10^{-4}–3 erg/cm^2 for large phospholipid bilayer vesicles and 10^{-2}–10 erg/cm^2 for red blood cells.

INTRODUCTION

Recently, an experimental method and analysis that provides a means to measure the affinity of red cell membranes for other surfaces was introduced (Evans, 1980). The concept is to use the elastic deformation of the red cell membrane as a "transducer" for measuring the interfacial free energy density which represents the work required to form the adhesive contact. The experimental procedure is to aspirate a flaccid red cell with a micropipet and manipulate the aspirated cell close to the surface to be tested without forcing the surfaces to contact. Then, the micromanipulators are left stationary. Because of the Brownian motion of the flaccid cell surface, eventually the membrane forms contact and the adhesion occurs. The deformation of the red cell membrane and its measured elastic properties are used to determine the work of adhesion. The derivative of the work of adhesion with respect to an increase in contact area yields the interfacial free energy density that quantitates the affinity of the cell membrane for the "test" surface. The approach has been used to study the affinity of red cell membranes for each other as mediated by high molecular weight dextran molecules (Buxbaum, 1980) where the interfacial free energy densities are in the range 10^{-4}–5×10^{-3} erg/cm^2. These values represent the useful range for the flaccid red cell technique. The upper bound is determined by the surface elastic shear modulus of the red cell membrane (Evans, 1980). For surface affinities characterized by interfacial free energy densities much greater than the red cell membrane elastic shear modulus (6.6×10^{-3} dyn/cm [Waugh and Evans,

1979]), the red cell membrane behaves in a manner mechanically analogous to a phospholipid bilayer vesicle with the membrane force resultants dominated by isotropic tension. The maximum interfacial free energy density which can be measured is determined by the isotropic tension that produces lysis, i.e., 5–10 erg/cm^2 (dyn/cm). Thus, for the range 10^{-2}–10 erg/cm^2, the analysis of membrane deformation produced by surface adhesion will be common to both red cells and large phospholipid bilayer vesicles, which is the subject to be considered in this article.

Mechanics of Large Vesicle Adhesion

Fig. 1 is a schematic of the experimental procedure. A large vesicle or red cell is aspirated by a micropipet with sufficent suction pressure to form a spherical segment outside the pipet (e.g., with a pipet of 10^{-4}-cm radius, the suction pressure required to force the red cell into this shape is ~500–1,000 dyn/cm^2). The phospholipid bilayer vesicle must be reduced in volume osmotically to have excess surface area whereas the red cell must be slightly swollen osmotically to reduce its surface area to volume ratio. Next, the vesicle is brought into close proximity of the surface to be tested (shown in Fig. 1 as either another sphere or a flat surface). Then, the suction pressure is reduced to permit adhesion and the new equilibrium configuration is established. In contrast to a liquid drop with a free interface, the vesicle or red cell is constrained to have constant surface area and constant internal volume (to <1% variation) for the range of tensions up to dyn/cm. These constraints plus the fixed distance from the pipet to the test surface provide for the stable equilibrium of the membrane at intermediate positions in the pipet with specific contact areas for adhesion. The mechanical analysis of the equilibrium shape provides the ratio of the interfacial free energy density for the surface affinity to the pipet suction pressure. Even though the membrane mechanical

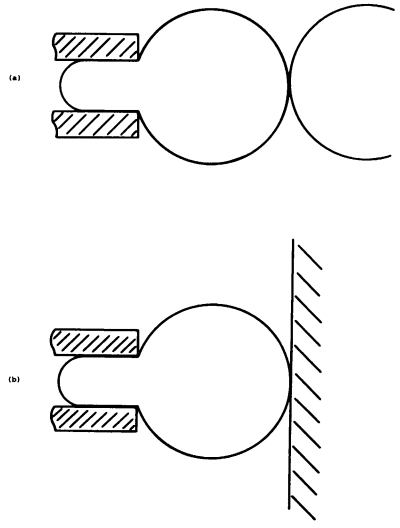

FIGURE 1 Schematic of experimental procedure for measuring the affinity of vesicular membranes for other surfaces. A large vesicle or red cell is aspirated into a micropipet with sufficient suction pressure to form a spherical capsule outside the pipet, then manipulated into close proximity of the surface to be tested (shown here as either a rigid sphere or flat surface). Finally, the suction pressure is reduced to permit adhesion.

behavior and analysis are different, the approach is similar to that introduced by Derjaguin and coworkers in 1939 for gas bubbles pressed against solid surfaces (Blake, 1975).

Most surface reactions (including the electrostatic and electro-dynamic interactions of colloidal systems) are really prominent only over short range (e.g., a few hundred ångströms) compared to measurable cellular dimensions. Thus, the details of the actual forces between the surfaces are not observable and are cumulated into an integral of force times displacement, i.e., the work involved in formation of adhesive contact. Since the surface radii of curvature are in general much larger than the distance between surfaces, the forces are distributed per unit surface area as a normal traction, σ_z; therefore, the work per unit area involved in formation of the adhesive contact is the integral relation:

$$\gamma = -\int_{\infty}^{z_0} \sigma_z \, dz, \tag{1}$$

where the contact position, z_0, refers to the equilibrium position for membrane surfaces that have adhered. The distance, z, is measured from a surface that is rigidly fixed to the surface that is displaced from a position considered to be initially "far away."

Near the equilibrium state, the virtual work done on the cell capsule is the integral over the capsule of the surface tractions times the virtual displacement of the surface. Since only uniform pressure exists in the fluid phases, this integral can be expressed in terms of the external fluid pressure, p_0, the pressure in the pipet, p_p, and the interfacial energy density, γ, for the adhesion process:

$$\delta W = -P_0 \int_0 (\delta\zeta_n) dA - P_p \int_p (\delta\zeta_n) dA + \gamma \cdot \delta A_c,$$

where the integrals represent the cell segments outside and inside the pipet, respectively; $\delta\zeta_n$ is the displacement normal to the surface; and A_c is the interfacial contact area. (Interaction with the pipet wall is assumed to be negligible.) The integrals are simply the virtual changes in the volumes of the cell segments outside and inside the pipet, respectively. Since the total volume of the cell is constant, these volume variations are equal but of opposite sign; thus,

$$\delta w = \Delta P \cdot \delta v_p + \gamma \cdot \delta A_c,$$

where $\Delta P \equiv P_0 - P_p$. The variation of the volume in the pipet is given by the variation of the projection length, L, in the pipet for lengths greater than the pipet radius, R_p; consequently, the variation in work is given by the virtual displacement of a pipet suction force plus the interfacial energy density times the variation in contact area:

$$\delta W = (\pi R_p^2) \Delta P \cdot \delta L + \gamma \cdot \delta A_c. \tag{2}$$

For an isothermal, equilibrium process, the variation in work is equal to the variation in Helmholtz free energy of the membrane plus the interior contents. The membranes of phospholipid bilayer vesicles and red cells exhibit a great resistance to area dilation or condensation (Evans and Hochmuth, 1978; Evans and Waugh, 1977; Kwok and Evans, manuscript in preparation). Also, because of the ionic strength of internal and suspending media, these globules greatly resist volume changes. Consequently, the vesicle geometry can be considered to be constrained to have constant surface area and constant volume. Thus, in this situation where the shear elasticity is negligible, the membrane mechanical equilibrium is

maintained by an isotropic tension, $\bar{T}$, which is related to the internal pressure and the mean curvature of the surface. The change in free energy of the membrane and internal contents produced by the deformation can be neglected; therefore, equilibrium is established when the variation in work, Eq. 2, is equal to zero;

$$\delta W = 0. \tag{3}$$

From this relation, the interfacial free energy density is found to be the product of the pipet suction force times the derivative of the aspirated length with respect to the increase in area of adhesive contact (Evans, 1980):

$$\gamma = -\Delta P(\pi R_p^2)\left(\frac{dL}{dA_c}\right). \tag{4}$$

The geometric factor, (dL/dA_c), is determined by the constant volume and area constraints plus the mechanical equilibrium requirement that the mean curvature of the unsupported membrane outside the pipet is constant, i.e.,

$$\frac{1}{R_1} + \frac{1}{R_2} = \text{constant}, \tag{5}$$

where R_1 and R_2 are the principal radii of curvature for the surface.

For an axisymmetric surface, Eq. 5 can be written as a differential equation and integrated to give an expression for the included angle, θ, between the outward surface normal and the axis of symmetry:

$$\sin\theta = \frac{1}{r}\left[\frac{(R_0 - R_1\sin\theta_1)r^2 + (R_1R_0)(R_0\sin\theta_1 - R_1)}{R_0^2 - R_1^2}\right], \tag{6}$$

where r is the radial distance from the axis of symmetry R_0 is the equatorial radius (where $\sin\theta \equiv 1$) and R_1 and θ_1 are the radius and angle values for the location where the contour begins (i.e., either at the pipet entrance or the contact surface). Since Eq. 6 is symmetric relative to the equatorial plane, the following relation exists between the radius and angle (R_p, θ_p) at the pipet entrance and the radius and angle (R_a, θ_a) at the contact surface:

$$\frac{(R_0 - R_p\sin\theta_p)}{(R_0^2 - R_p^2)} = \frac{(R_0 - R_a\sin\theta_a)}{(R_0^2 - R_a^2)}. \tag{7}$$

From differential geometry, it is recognized that the spatial coordinates (r, z) are related to the angle, θ, by

$$\left|\frac{dz}{dr}\right| = \tan\theta. \tag{8}$$

Hence, with Eqs. 6 and 7, Eq. 8 can be integrated numerically to provide the geometry of the unsupported outer surface. The numerical solution involves iteration to satisfy the constant surface area and constant volume constraints for specific values of the distance, z_c, between the pipet entrance and the contact surface. The result is a unique relation between the aspirated length and the area of contact for the particular pipet, vesicle, and test surface configuration.

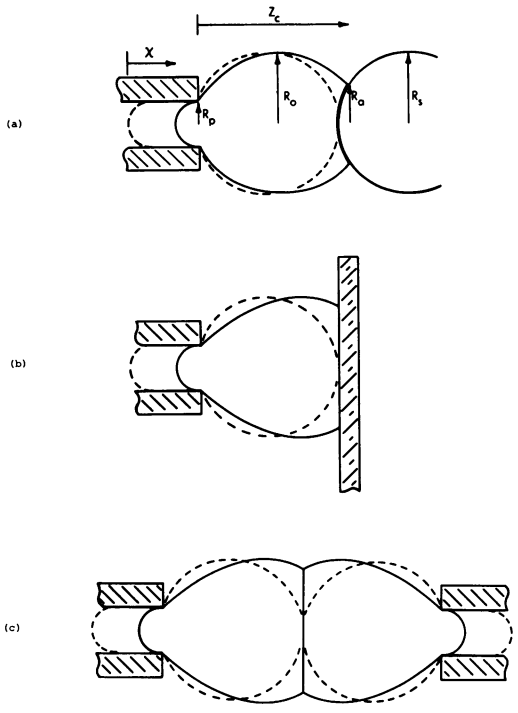

FIGURE 2 Equilibrium contours for vesicle adhesion to: (*a*) rigid spherical surface, (*b*) flat surface, (*c*) another vesicle of equivalent size aspirated by the same suction pressure. The particular adhesive contact shown here was sufficient to produce a displacement of the aspirated projection equal to two pipet radii.

RESULTS AND DISCUSSION

Results will be given for two cases: (*a*) the adhesion of a vesicle to a rigid spherical surface; and (*b*) the adhesion of a vesicle to a flat surface (also equivalent to the symmetrical adhesion of two vesicles). It is convenient to normalize all dimensions by the pipet radius, R_p, and to use the displacement, x, of the aspirated projection. With these conventions, Eq. 4 is given by

$$\gamma = \pi \Delta P \cdot R_p \cdot \left(\frac{d\tilde{x}}{d\tilde{A}_c}\right),$$

where $\tilde{x} \equiv x/R_p$ and $\tilde{A}_c \equiv A_c/R_p^2$. For both of the calculations, the aspirated vesicle had an initial outer diameter equal to six pipet radii and an initial aspirated length equal to three pipet radii as shown in Fig. 1.

Fig. 2 shows the equilibrium contours for these cases where the required contact area produced a displacement equal to two pipet radii. The equilibrium relationship between the dimensionless displacement, $\tilde{x}$, and the dimensionless contact area, $\tilde{A}_c$, is plotted in Fig. 3 for the rigid spherical contact surface and the flat contact surface. The derivative of the dimensionless displacement with respect to dimensionless contact area gives a dimensionless form of the interfacial free energy density $\gamma/\pi\Delta P \cdot R_p = d\tilde{x}/d\tilde{A}_c$. The derivative, $(d\tilde{x}/d\tilde{A}_c)$, is plotted in Fig. 4 for each case as a function of the dimensionless contact area, $\tilde{A}_c$. It is apparent that the flat surface will provide greater sensitivity for the affinity measurement because the derivative range is $0.0 < d\tilde{x}/d\tilde{A}_c < 0.17$. By comparison, the derivative range for

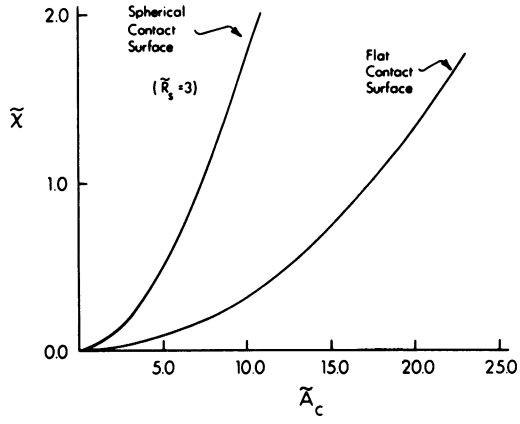

FIGURE 3 The equilibrium relationship between the displacement of the aspirated length and the contact area. The displacement in normalized by the pipet radial dimension and the contact area by the square of the pipet radial dimension.

the particular spherical surface shown in Figs. 1 and 2 is $0.0 < d\tilde{x}/d\tilde{A}_c < 0.33$. For a commonly used micropipet radius of 10^{-4} cm, the range of interfacial free energy densities would be given by $0.0 < \gamma/\Delta p < 5 \times 10^{-5}$ (flat contact surface) and $0.0 < \gamma/\Delta p < 10^{-4}$ (spherical contact surface), where the suction pressure is in units of dyn/cm^2 and the interfacial free energy density is in units of erg/cm^2.

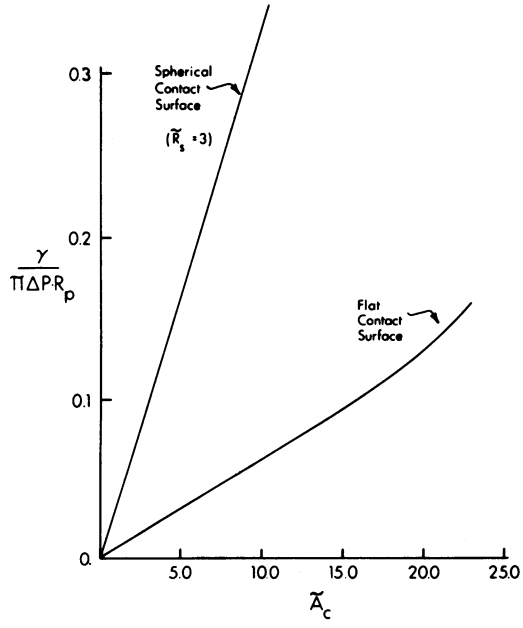

FIGURE 4 The derivative of the displacement of the projection length with respect to the contact area is plotted vs. the contact area. All dimensions are normalized by the pipet radius. The ordinate is the dimensionless value of the interfacial free energy density, $\gamma/(\pi\Delta p \cdot R_p)$, which represents the surface affinity.

For a large phospholipid bilayer vesicle, the aspiration pressure can be as low as 10–100 dyn/cm^2 which implies a sensitivity for measurement of surface affinity on the order of 10^{-4}–10^{-3} erg/cm^2. The maximum interfacial free energy density that can be measured is limited by the tension at lysis, 3 erg/cm^2. As previously discussed, the red cell can be modeled by this analysis when the isotropic tension exceeds the membrane shear resultant; for a 10^{-4}-radius suction micropipet, suction pressures >500–1,000 dyn/cm^2 easily satisfy this condition. Thus, the analysis can be used for red cell membrane affinity measurements in the range 10^{-2}–10 erg/cm^2.

The author is grateful to his colleague Robert Hochmuth for the correction of an error in the original computational results.

This work was supported in part by National Institutes of Health grants HL 16711 and HL 24796.

Received for publication 22 February 1980 and in revised form 18 April 1980.

REFERENCES

BLAKE, T. D. 1975. Investigation of equilibrium wetting films of *n*-Alkanes on α-alumina. *J. Chem. Soc. Faraday Trans.* I. **71**:192–208.

BUXBAUM, K. L. 1980. Measurement of the intrinsic affinity of red cell membranes for other membrane surfaces. Ph.D. Dissertation, Duke University, Durham, N. C.

EVANS, E. A. 1980. Minimum energy analysis of membrane deformation applied to pipet aspiration and surface adhesion of red blood cells. *Biophys. J.* **30**:265–284.

EVANS, E. A., and R. M. HOCHMUTH. 1978. Mechano-chemical properties of membranes. **10**:1–64.

EVANS, E. A., and R. WAUGH. 1977. Osmotic correction to elastic area compressibility measurements on red cell membrane. *Biophys. J.* **20**:307–313.

WAUGH, R., and E. A. EVANS. 1979. Thermoelasticity of red blood cell membrane. *Biophys. J.* **26**:115–132.