Specific adhesion of vesicles to compliant bio-adhesive substrates

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1. Introduction

Cell behavior is mediated by variety of physiochemical properties of extracellular matrix (ECM). Surface chemistry (Liu et al., 2007; Morgenthaler et al., 2008), roughness (Martin et al., 1995; Lampin et al., 1998) and distribution pattern of cell adhesive proteins (Chen et al., 1998; Christman et al., 2006; Cavalcanti-Adam et al., 2007) are among the ECM properties which are known to modulate various cellular physiological functions. Mechanical stiffness of ECM is also found to be a major regulator for multiple aspects of cellular function, ranging from cell motility to the lineage commitment and differentiation (Discher et al., 2005). Quantification of migration speed of motile cells cultured on substrate with variable stiffness reveals a biphasic dependence on substrate compliance, suggesting the existence of an optimal stiffness capable of supporting maximal speed of migration (Peyton and Putnam, 2005). Contractile cells, such as vascular smooth muscle cells (VSMC), become more proliferative and less apoptotic in response to the increasing the substrate stiffness (McDaniel et al., 2007). Other studies have demonstrated the strong influence of substrate elasticity on the lineage commitment of naive stem cells and driving their differentiation to variety of mature cells (Engler et al., 2004a, 2006).

The acute molecular basis of the sensory mechanism through which the tissue cells sense the ECM elasticity and translate it into a downstream response is largely unknown. It is currently believed that the downstream signaling in response to the matrix stiffness should be started at the molecular level and by transmembrane integrin receptors present on the surface of adherent cells. These mobile proteins can selectively associate with the complementary adhesive ligand molecules of ECM, providing not only the adhesion between the cell membrane and the matrix, but also a pathway of force transmission from inside the cell to the elastic substrate. The cytoskeletal force exerted on the ligand–receptor anchorage sites can deform the soft substrate and trigger the action of signaling molecules and mechano-transducers (Schwarz and Bischofs, 2005). Considering the role of these receptor-mediated linkages in initiation of the elastosensitivity, one could expect that substrate stiffness may also regulate the state of cellular adhesion. Pelham and Wang (1997) in a seminal study reported that the adhesion of rat kidney epithelial and 3T3 fibroblastic cells are strongly regulated by the rigidity of the underlying collagen coated polyacrylamide substrates. Subsequent and more quantitative works with different elastic substrates showed that cells in general, exhibit an apparent adhesion preference for stiffer substrates with a more organized cytoskeleton and a larger but saturable projected spread area with increasing the substrate stiffness (Lo et al., 2000; Engler et al., 2004b).

How stiffness couples with adhesiveness of the substrate to upregulate the cellular adhesion is a question raised recently with...
the suggestion that surface elasticity and adhesiveness are perhaps the orthogonal determinants of similar importance in a host of similar responses (Geiger, 2001). For example, measurement of spreading area of VSMCs on collagen coated poly(methyl methacrylate) gels shows that on relatively stiff substrates cells exhibit a strong biphasic dependence to the changes in collagen density, whereas on soft substrates their limited spreading is much less sensitive to the density of collagen (Fig. 1). The direction of lineage specification of stem cells is also been demonstrated to depend not on the substrate stiffness alone, but more on a definitive combination of stiffness and adhesiveness of the substrate (Rowlands et al., 2008).

The main purpose of the present contribution is to investigate the effect of relevant physical parameters on the cell adhesion to a compliant bio-adhesive substrate. Vesicles reconstituted with receptor proteins are regularly used as useful model systems to study the physics of bio-adhesion. In this paper, we elucidate the theoretical bases of the interplay between the substrate stiffness and the vesicle adhesion, using an equilibrium thermodynamic model which is originally developed by Bell et al. (1984) and Dembo and Bell (1987), hereafter referred to as D–B model. Within this framework, the substrate-vesicle equilibrium contact is assumed to ensure minimization of the free energy contributed by different components describing the energetic and the entropic nature of the interaction between the membrane and the substrate. Recent studies on controlled adhesion of biomimetic vesicles have reasoned the success of the equilibrium thermodynamic models, suggesting that cell adhesion can be envisioned as first-order wetting transition of the bilipid membranes (Sackmann and Bruinsma, 2002). Here the term membrane refers to a very thin and highly flexible layer which encloses the vesicle. For the modeling purpose, this layer is pictured as a thin elastic shell with a finite bending rigidity. The shape of the membrane therefore is controlled by the interplay between the adhesion and its elastic deformation. In the theoretical analysis to follow, we generalize the D–B model to include the effect of substrate compliance and examine whether such a purely physical picture of bio-adhesion is able to describe some of the experimentally observed features of cell adhesion to compliant substrates.

2. Model description

Fig. 2 shows the schematic representation of a vesicle in contact with a compliant substrate S with total surface area of $A_S$. The surface of the isotropic elastic substrate is coated by surface conjugated ligands with constant concentration of $n_L$. Vesicle membrane, represented by closed surface $M$, has the surface area of $A_M$ and carries a constant number of laterally mobile receptors with total surface density of $n_R = N_R/A_M$, where $N_R$ is the total number of the receptors. The transmembrane mobile receptors have specific affinity for the complementary immobilized ligands on the substrate. The non-covalent association of ligands with receptors takes place within a subset of $M$ and $S$, henceforth denoted as the contact zone $C$ with surface area of $A_C$. The free mobile receptors are assumed to be uniformly distributed over the membrane at thermodynamic equilibrium, consistent with the requirement of maximum entropy. Hence, the concentration of free receptors is

$$n_M = \frac{N_R - N_C}{A_M},$$

where $N_C$ is the number of ligand–receptor bonds within the contact zone. The concentration of free ligands in the contact zone can be expressed as

$$n_L = \frac{N_L - N_C}{A_C},$$
\[ n_l = n_s - n_c. \] (2)

Here \( n_s = N_s/\Lambda_c \) shows the concentration of ligand–receptor bonds.

The vesicle, substrate, and surrounding medium are viewed as a thermodynamic system in equilibrium, with constant volume, pressure, and temperature. We assume that the primary cause for a change in energy of the system is the establishment of ligand–receptors bonds. However, there are some energy barriers to overcome before the ligand–receptor binding can occur. Surface receptors of cells are generally buried deep within a hydrated layer of oligosaccharides such as glyocalyx chains with a thickness up to tens of nanometers. The resulting non-specific repulsion due to the steric interactions, electrostatic potentials, and hydration of glyocalyx conspire to produce an energy barrier to cell-substrate adhesion. The resulting energy penalty (per unit area) for compressing the glyocalyx to the height \( \Delta \) is often represented by the following phenomenological function (Bongrand and Bell, 1984)

\[ \Gamma(\Delta) = \frac{\tau}{\Delta} \exp(-\Delta/\gamma). \] (3)

Here, the parameters \( \tau \) and \( \gamma \) measure the thickness and stiffness of glyocalyx layer on the membrane. The surface of the cell mimetic vesicle in our study is assumed to be coated with a brush layer of glyocalyx molecules, showing the repulsive potential shown by Eq. (3).

Upon establishment of the adhesion between a ligand and a receptor, the bonded molecules are being stretched by the interfacial repulsion which tends to separate the membrane from the adhesive substrate. Forces exerted by the stretched bonds locally deform the membrane and compliant substrate. The separating distance between the membrane and substrate changes along the contact zone due to the adhesion-induced deformations. The detailed analysis of these local deformations although is possible, but leads to considerable complexity. Here, for simplicity, let us assume that \( \Delta \) is the average distance between the substrate and (undeformed) membrane (Fig. 2). The stressed bonds pull the compliant substrate leading to a local deformation of \( \Delta \) at each bonding site. This adhesion induced deformation and the tensile force of each bond, \( F_b \), are connected as \( F_b = k_S \sigma \), where (Kendall, 1971)

\[ k_S = \frac{E_S d}{1 - v_S^2}. \] (4)

Here \( d \) is the diameter of a bond and \( E_S \) and \( v_S \) are the Young’s modulus and the Poisson’s ratio of the elastic substrate. If the stiffness of the membrane and ligand–receptor bonds are modeled by spring constants \( k_M \) and \( k_c \), then we have \( k_M (\Delta - L) = k_c \), where \( L \) shows the length of an unstrressed ligand–receptor bond and \( k_c = (k_M + k_L)^{-1} \).

In order to conform to the flat geometry of the substrate, local deformation of lipid bilayer around the contact zone is necessary. This deformation occurs predominantly by elastic bending. The finite bending stiffness of the membrane prevents the formation of sharp edges at the boundary of the contact zone and leads to a smooth transition from a free to an adhering membrane. The elastic energy of this deformation can be accounted for simply by consideration of the membrane as a soft elastic shell and by mechanical analysis of the adhering membrane near the substrate which is subjected to in-equilibrium forces. Fig. 3 shows a schematic view of the membrane near the edge of the contact zone. The local contribution to bending energy scales with the square of the local curvature. Therefore, the bending energy is concentrated along the boundary of the contact zone and can be approximately neglected elsewhere. On the basis of elementary bending theory (Bruinsma, 1996), one can show that the membrane profile \( u(r) \) follows:

\[ \kappa \frac{\partial^2 u}{\partial r^2} - \frac{\partial^2 u}{\partial r^2} = 0, \] (5)

where \( r \) is the local axis perpendicular to the contact line, \( \kappa \) is the bending modulus, and \( \sigma \) is the outer surface tension (outside of the contact zone) which is taken to be a constant (Coombs et al., 2004). The corresponding boundary conditions are \( u(0) = 0, u'(0) = 0 \), and \( u'(\infty) = \sigma \), where \( \sigma = \cos^{-1} \sqrt{1 - \frac{4k}{\kappa}} \) is the macroscopic contact angle (Fig. 2). A solution satisfying these boundary conditions is

\[ u(r) = \frac{\sigma}{\kappa} \left[ 1 - \exp(-r/\lambda) \right], \quad \text{for} \ r > 0, \]

\[ 0, \quad \text{for} \ r \leq 0, \] (6)

where \( \lambda = \sqrt{\kappa/\sigma} \) is the capillary length. The elastic energy of bending per unit length of the contact boundary is \[ \frac{\sigma^2}{2} \int_0^\lambda \left( \frac{\partial u}{\partial r} \right)^2 \, dr \] (see Coombs et al., 2004).

According to D–B thermodynamic model (Bell et al., 1984; Dembo and Bell, 1987), the establishment of stable equilibrium adhesion warrants the minimization of the change in Gibbs free energy of the system. In the thermodynamic paradigm model of adhesion, depicted by Fig. 2, the change in Gibbs free energy of the system is constituted from: (1) the ligand–receptor bond formation restricted to the contact zone, (2) the interfacial repulsion between the cell and the substrate mediated by the bulky macromolecules on cell surfaces, (3) the deformation of binding molecules to alleviate the repulsion, (4) the substrate and the membrane deformation, and (5) the elastic bending of membrane, among which the last two components are added to what has been originally outlined in the D–B model. In what follows, we drive an expression for the Gibbs free energy of the thermodynamic system shown by Fig. 2.

3. Minimization of free energy

Taking into account all different contributions involved in the adhesion, the change in Gibbs free energy of the system can be represented by

\[
G = \int_{x=M} n_M(x) \mu_M(x) \, dx + \int_{x=S} n_S(x) \mu_S(x) \, dx \\
+ \int_{x=C} n_C(x) \mu_C(x) \, dx + \int_{x=C} \Gamma(\Delta) \, dx + \frac{N_s}{2} k_S \sigma^2 \\
+ \frac{N_s}{2} k_c (\Delta - L)^2 + \sqrt{\pi A_c} \int_0^\infty \kappa \left( \frac{\partial^2 u}{\partial r^2} \right)^2 \, dr, \] (7)
leads to a set of non-algebraic equations which can be solved numerically.

4. Results and discussion

In this section, we implement the proposed model to see how changing the compliance of an adhesive substrate affects the vesicle configuration in equilibrium. Following D–B model, we describe the vesicle configuration in terms of three key parameters \( A, N_c, \) and \( A_c \). The goal is to study the variation of these configurational variables by changing the substrate compliance concomitant with other control parameters, such as ligand density, receptor-ligand energetic affinity, substrate repulsion, and surface tension. Table 1 represents the values of dimensional model parameters and their range of variation based on the theoretical predictions or experimental measurements. The following dimensionless parameters have been used in our analysis: \( \frac{A_c}{A}, \frac{n_l}{n_R}, \frac{N_c}{N_L}, \frac{1}{A/L}, \) and \( k_S = k_S/k_c \).

We begin by investigating that how substrate rigidity couples with ligand density to modulate the vesicle configuration. Fig. 4(a) and (b) shows the variation of the vesicle spread area \( \langle A_c \rangle \), as the compliance \( (k_1) \) and adhesivity \( (n_h) \) of the substrate change. The curves shown in Fig. 4(b) are cut through the curved surface in three dimensions shown by Fig. 4(a). The first notable observation is the biphasic dependence between the spread area and the ligand concentration. On relatively stiff substrates, the spread area of the vesicles exhibits strong biphasic dependence to the changes in ligand density and acquires a large value on an optimal intermediate ligand density. On softer substrates, the vesicle flattens to a smaller spread area and the biphasic dependence between the area and ligand density are less pronounced. These results, to some extent, are reminiscent of the spreading curves of smooth muscle cells on collagen coated polyacrylamide gels, reported by Engler et al. (2004b), as shown in Fig. 1. At very large concentration of ligands, the contact area approaches asymptotic value, irrespective of the compliance of the substrate. Below a certain critical concentration of surface ligands however, the vesicle adhesion may not be thermodynamically admissible. This critical concentration is found to depend on the compliance of the substrate as shown by Fig. 5.

Although the general biphasic nature of the experimental results noted in Fig. 1 is predicted by model, the character of the response for lower ligand densities is quite different. It is known that the state of weak adhesion is dominated by the contribution of non-specific adhesions (Seifert, 1997) which are not included in the manifold of generic interactions considered in this paper. Cell adhesion involves both specific and non-specific adhesion where the latter is mediated by van der Waals forces competing with shorter-ranged electrostatic or hydration repulsions. Experimentally accessible values of non-specific adhesion lie in the range

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* Defined as \( K_\infty = \exp \left( \frac{\mu_0^\gamma + \mu_0^\mu + \mu_0^\kappa}{k_B T} \right) / k_B T. \)
$10^{-4}$–$10^{-6}$ J/m$^2$, which corresponds to membrane–substrate separations in the range 3–30 nm. Such relatively weak adhesion energy regulates the state of adhesion when the specific adhesions are weak. This is expected to be a reason underlying the difference between the model prediction of adhesion area and experimental observation at low ligand densities.

How substrate compliance couples with ligand density to modulate the morphology of adherent vesicles is rooted in the thermodynamic nature of interaction between the vesicle membrane and the substrate. If the initial ligand density is low (but still high enough to warrant the stable adhesion), increasing the ligand density leads to increasingly higher number density of ligand–receptor pairs within the contact zone and thereby larger contact area (Fig. 6). However, concurrent with increasing the number of ligand–receptor bonds, the gap distance $D$ between the vesicle and substrate gradually decreases (Fig. 7) leading to increasingly larger membrane–substrate repulsion (see Eq. (3)). As a result, the contact area acquires a maximum value at a certain ligand density, above which further increase in ligand concentration leads to no further increase or reduction of $A_C$.

At any binding point, the local deformation of the substrate caused by the pulling force of a stretched ligand–receptor bond can be approximated using $\delta = k_S(D - L)/k_S$. From Fig. 7, it can be observed that a large decrease in the substrate rigidity (more than one order of magnitude) leads to just a small reduction of the separation distance $A$. As a result, the compliant substrate must undergo a large deformation at each adhesion site which in turn leads to a significant increase in elastic energy of the substrate deformation. Therefore, the formation of new ligand–receptor bonds (thereby increasing the size of the contact zone) becomes increasingly costly as the stiffness of the substrate decreases. In our equilibrium analysis, the energy cost associated with the substrate deformation must be balanced by the enthalpy of
reaction between the receptors and ligands. Hence, on compliant substrates, the vesicle reduces its contact area to avoid the high energy penalty of the substrate deformation. This is the underlying mechanism which mediates the interrelation between the substrate stiffness and the size of the contact zone. The regulatory effect of substrate compliance is supposed to weaken on substrates with very high concentration of ligands due to significant receptor depletion, as shown by Fig. 4.

Fig. 4 shows that the vesicle contact area responds most strongly to changes in substrate stiffness at low $k_S$ whereas on stiffer substrates $A_C$ approaches saturation. This behavior can be controlled by changing other parameters such as the ligand–receptor binding affinity or membrane substrate repulsion. Fig. 8(a) shows the effect of changing the ligand–receptor binding affinity on the variation of $A_C$. This has been done by changing the value of equilibrium constants $K_{eq} = \exp\left[\left(\mu_R^C + \mu_L^C - \mu_C^C + k_BT\right)/k_BT\right]$, which is defined by analogy with classical equilibrium constants for solution phase reactants. The contact area on increasingly stiffer substrates approaches a plateau which depends strongly on the affinity constant $K_{eq}$. The contact area increases by enhancing the binding affinity between the receptors and the ligands, simply owing to increment of $C_c$ contribution in the free energy. In addition, changing the binding affinity significantly alters the critical substrate stiffness which is required to establish the equilibrium adhesion. As expected, the use of ligand–receptor pairs associated with a large binding constant leads to receptor depletion.

The effect of the repulsive potential on the contact area is shown by Fig. 8(b). In our model, the membrane–substrate repulsion is associated with the presence of glycocalyx–like molecules on the ventral side of the membrane. In order to form a close contact between the cells, the long chains repeller molecules forming the glycocalyx must be compressed. Reducing the gyration space to less than the gyration radius of the repelling polymers leads to a strong steric energy penalty for adhesion, represented by Eq. (3). Hence, incorporation of repelling molecules naturally leads to the reduction of effective adhesion strength.

The last control mechanism investigated is the surface tension of the vesicle. The effect of membrane tension on the size of the contact zone is found to be negligible in comparison with the detrimental contributions of other control parameters. Fig. 9 compares the vesicle’s contact area at different values of $\sigma$. Although in general, the area of the contact zone reduces with increasing $\sigma$, the difference between the relative values of $A_C$ is insignificant. This is rooted in the negligible contribution of bending energy in total free energy of the vesicle upon adhesion. Membrane tension controls the shape of the membrane profile at the boundary region around the contact zone and changes the elastic energy density of the deformed membrane. It is important to notice that $\kappa$ is negligible compared to the specific energy of adhesion. Consequently the bending contribution in free energy is very small (less than $10^{-5}k_BT$ per bond) in comparison with the energy of each ligand–receptor bond (of the order of $1 - 10k_BT$) (Smith and Seifert, 2005). As the number of bonds is very large, it is clear that the bending energy is not of comparable magnitude. Hence, the membrane deformation...
due to bending is energetically inexpensive and can be virtually omitted from the minimization of the free energy.

The vesicle adhesion is generally started with formation of tightly bound segregated receptors, analogues to the focal adhesion points in cell adhesion (Wiegand et al., 1998; Sackmann and Bruinsma, 2002). These sparse adhesion plaques slowly merge over a period of several hours, when vesicle acquires its (quasi) equilibrium configuration. The coarsening is predominantly driven by the line tension arising due to the bending deformation at the membrane (Sackmann and Bruinsma, 2002). Naturally, our equilibrium model cannot elucidate that how the substrate compliance regulates the growth and nucleation of these domains. Correct analysis of the spontaneous growth of the adhesion plaques warrants a kinetic model, similar to those proposed by Boulbitch et al. (2001) and Brochard-Wyart and de Gennes (2002). However, increasing the compliance of the substrate is expected to impede the growth of these transient domains due to the mechanism described in this paper: the reduction of substrate stiffness makes the thermodynamically driven process of growth costly and therefore impedes the spontaneous spreading of the membrane. Recent experimental study on myofibroblasts adhesion (Goffin et al., 2006) shows that in general, reduced stiffness of the bio-adhesive substrate correlates with the smaller size of the focal adhesions. The role of the thermodynamic contributions in regulation of the time dependent adhesion to a compliant substrate is considered in a separate paper (Sarvestani, 2010).

5. Conclusion

We have outlined a framework to investigate the thermodynamic equilibrium adhesion of a vesicle to a compliant substrate functionalized with immobilized bio-adhesive ligands. The vesicle is modeled as an enclosed soft elastic shell, reconstituted with mobile receptors and a repelling layer on the ventral side. The free energy function of the system is assumed to be comprised from the following contributions: the membrane–substrate repulsive potentials, stored elastic energy (in deformed membrane and substrate), binding enthalpy, and mixing entropy of mobile receptors. The ligand density is held constant at $n_l = 1000 \mu m^{-2}$.

with a biphasic dependence on the density of ligands. These predictions are reminiscent of the experimental observations of spread area of cells adhering on compliant bio-adhesive substrates. This is an interesting result considering the lack of contribution of intracellular signaling or actively regulated cytoskeleton in the proposed physical model for the adhesion. This suggests that the mechanistic pathways inherent to membrane–substrate thermodynamic interactions can be equally important as intracellular signaling pathways to mediate the process of rigidity sensing by cells.

Acknowledgments

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References


