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Physical Interpretation of the Maximum Receptor−Ligand Bond Spacing to Ensure Cell Adhesion in Ligand-Coated Substrates

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Recent experiments by Arnold et al. (Arnold, M.; Cavalcanti-Adam, E. A.; Glass, R.; Blummel, J.; Eck, W.; Kantlehner, M.; Kessler, H.; Spatz, J. P. *ChemPhysChem* **2004**, *5*, 383) revealed that a distance of less than 58−73 nm between receptor−ligand bonds is necessary to ensure focal adhesion in integrin-mediated cell adhesion on ligand-coated substrates. In this letter, we consider focal adhesion growth to be a process assisted by thermal fluctuations and receptor−ligand binding and resisted by repulsive "bulge pressure" and membrane deformation. By applying balance between these forces, we obtain a critical spacing of receptor−ligand bonds given as $2h[(\alpha k_B T/\beta E h^3)^{1/3}-(E/p)^5]^{1/14}$, above which the growth of focal adhesion becomes difficult. Here h and E are the in-plane modulus and thickness of a cell membrane, respectively, p is a repulsive "bulge pressure" between the cell membrane and substrate, and α and β are constants on the order of 1. We use typical values of E and h for cell membranes and obtain the critical spacing of receptor−ligand bonds of around 39−89 nm for a wide range of repulsive bulge pressure.

1. Introduction

To achieve a wide variety of biological phenomena, the ability of cells to contact effectively and interact specifically with neighboring media plays a central role.[1,2] It is known that cells can sense the chemical and mechanical properties of surrounding systems[3−11] and regulate their adhesion and movement through binding protein molecules within cell membranes. Recent observation by Arnold et al.[12] revealed that a spacing of receptor−ligand bonds of less than 58−73 nm is critical to ensuring stable adhesion for a variety of cultured cells in ligand-coated substrates. At larger spacing, they observed that focal adhesion is difficult to develop and hence cell adhesion is not stable. Motivated by those striking experimental observations, we perform a dimensional analysis and give a formula for the maximum length of receptor−ligand bonds to ensure focal adhesion between cells and ligand-coated substrates. We consider focal adhesion growth to be a process assisted by thermal fluctuations and receptor−ligand binding and resisted by repulsive "bulge pressure" and membrane deformation. The critical spacing is an outcome of the balance between these forces.

The dependence of integrin-mediated cell adhesion on the spacing of bonding sites in ligand-coated substrates was indeed foreseen by Bell et al.[13,14] in their equilibrium thermodynamic framework of receptor−ligand binding. In Bell's theory, cell adhesion is a competition between specific attractions and nonspecific repulsions. The former is due to specific receptor−ligand interaction. Several possibilities may account for the nonspecific repulsive forces, including electrostatic interactions, osmotic interactions, glycocalyx repulsion, and so on. [14] To quantitatively explain the critical spacing of 58−73 nm between bonding sites to ensure effective bonding, Lin et al.[15,16] envision dynamic interactions between cell membranes and substrates as a compliant elastic membrane undergoing thermal undulation; the tendency for receptors in a cell membrane bonding with a ligands in a substrate is represented by an interaction potential. The critical spacing of ligand bonds in their model is the outcome of the competition between the thermal motion of the cell membrane and the free-energy reduction associated with bonding. The theoretical model leads to estimates of the bonding site span of less than 43−172 nm[16] to maintain stable focal contact. The opposite mechanism to adhesion in the model[15] is attributed to the thermal motion of the cell membrane, which has to be suppressed to ensure stable focal contacts.

Before the formation of focal contacts, thermal undulation is probably the driving force to bring receptor proteins in a cell membrane close to ligands in a substrate. The scenarios may be depicted as follows: (1) receptors and ligands form molecular bonds in a random fashion at the beginning when a cell membrane approaches a substrate, and these bonds are essentially nuclei of possible focal contacts; (2) thermal undulation helps to overcome "energy barriers", which may be set by the repulsive bulge pressure and membrane deformation before the formation of a bound complex, and allow new molecular bonds to form around a nucleus before it breaks; (3) the capability of the contact zone to spread during its lifetime controls the formation of a focal contact. Indeed, experiments by Capo et al.[17] revealed that cells must frequently be forced into close contact by centrifugation before strong bonding occurs. Although cell−cell adhesion was studied by Capo et al. and cell−substrate adhesion is modeled here, the energy barrier observed in cell−cell interaction should

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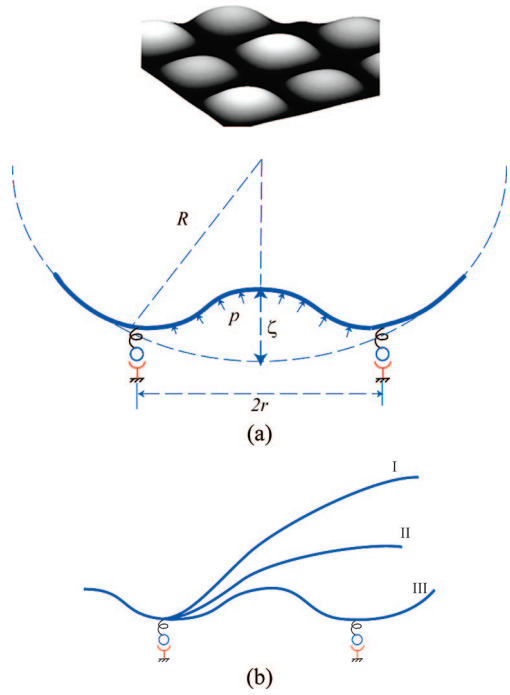

Figure 1. Diagrams of the interaction between a membrane and a substrate: (a) A portion of the idealized 3D morphology of a membrane is shown at the top, and the bottom shows the cross section of a 3D bulge induced by receptor−ligand bonds and the resultant repulsive bulge pressure p. The bulge (amplified) is assumed to appear in a large sphere with radius R, ζ is the out-of-plane deflection (the depth of the bulge), and $2r$ is the ligand spacing. (b) Envisioned bonding processing driving by thermal fluctuation. Given one site being bound, thermal vibrations bring the membrane close to a neighboring site (from I to II and then to III). The thermal energy in this process is comparable to the stored bending and stretching energies in the membrane.

be also expected to exist in cell−substrate adhesion. Moreover, it was shown by Grinnell[3] that centrifugation could bypass several metabolic steps otherwise required for the onset of bonding.

Therefore, there seem to be at least two conditions to be satisfied to initiate and maintain stable cell adhesion: (1) first, there is an energy barrier to be overcome for the spreading of a contact region; this energy barrier originates from the elastic energy difference while a cell membrane changes from one configuration to another to ensure bonding. For example, a membrane should be brought close to a substrate such that receptors and ligands are able to "shake hands". In the experiments of Capo et al.,[17] centrifugation was probably the driving force to overcoming the energy barrier. Thermal undulation is another possible driving force in the absence of external forces. (2) Second, as Lin et al.[15,16] have shown, the competition among energies by specific bonding, nonspecific repulsion, and thermal undulation is crucial to maintaining a stable focal adhesion. Both conditions will give rise to constraints on receptor−ligand spacing. The final critical spacing to ensure stable focal adhesion may be the intersection of the results from these two circumstances.

In what follows, we focus on the dynamics associated with the onset of cell adhesion and the spreading of a contact region and carry out a dimensional analysis on the process of focal adhesion growth during cell adhesion, which is assumed to be assisted by thermal fluctuations and receptor−ligand binding and resisted by repulsive bulge pressure and membrane deformation. We formulate the maximum spacing between ligand sites to ensure focal adhesion growth for a variety of cultured cells in a ligand-coated substrate.

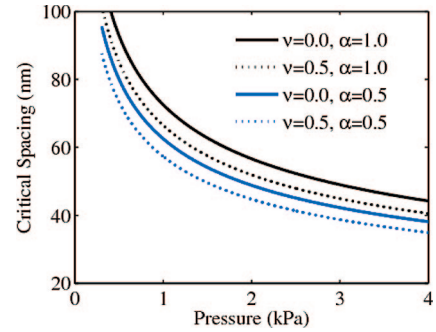

Figure 2. Dependence of critical spacing on the repulsive bulge pressure with two Poisson ratios ν (0, 0.5) at two α values (0.5, 1). The critical spacing is in the range of 39−89 nm for repulsive bulge pressure in the range of 0.5 to 3.0 kPa.

2. Methods and Results

The interaction between cells and ligand-coated substrates is idealized as a spherically shaped membrane on a flat, rigid surface. The idealized surface morphology of the membrane in contact with the substrate is shown at the top of Figure 1a. The deformation in membranes is analogous to that in curved plates. We note that the tensile stress created by stretching in a membrane is in general larger than that induced by bending.[18] Because of the dynamic nature of a cell membrane immersed in a thermal bath, membrane stretching and bending occur concurrently. Stored bending energy φ_b and stretching energy φ_s are given as $\varphi_b \approx Eh^3\zeta^2/l^4$ and $\varphi_s \approx Eh\ \zeta^4/l^4$, respectively,[18] where E is the modulus, h is the membrane thickness, l is the in-plane dimension of the membrane (equivalent to $2R$ in Figure 1a), and ζ is its out-of-plane deflection. A quick magnitude comparison between φ_b and φ_s tells us that neglecting the bending energy φ_s is valid only if $\zeta^2 \ll h^2$.[18] However, φ_b is negligible in the case of $\zeta^2 \gg h^2$. More likely, none of these conditions will be satisfied for membrane−substrate interactions with bonding spacing of 58−73 nm[12] and an effective membrane thickness of ~5 nm.[21] Considering energy contributions from both bending and stretching in the membrane during the initiation of focal adhesion, the bulge has a total elastic energy of[18]

$$\phi = \frac{1.2}{(1-\nu^2)^{3/4}}\frac{Eh^{5/2}\zeta^{3/2}}{R} \quad (1)$$

where ν is the Poisson ratio. In the case, that the bulge is induced by a uniform bulge pressure p, and the height of the bulge is related to the bulge pressure in the fashion of[18]

$$\zeta = \frac{h^5E^2}{R^4p^2} \quad (2)$$

With the simple geometrical condition (Figure 1a), the radius of the bulge in the contact interface is

$$r = \sqrt{R^2-(R-\zeta/2)^2} \approx \sqrt{\zeta R} \text{ for } R \gg \zeta \quad (3)$$

To initiate bonding, the total energy required to generate such a bulge is assumed to be within reach of the thermal fluctuation. The argument is based on the envisioned bonding process diagramed in Figure 2b. Whereas one site is bound randomly, the thermal fluctuation is responsible for bringing the membrane close to the neighboring bonding sites and making the neighboring bonding feasible. The thermal energy should be comparable to the energy stored in the bulge induced by the resultant repulsive bulge pressure when the membrane and the substrate move closer. Thus,

$$\phi = \alpha k_B T \quad (4)$$

where α is a constant depending on the modes of thermal fluctuation, which could be several halves.[15] In the following discussion, we

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take $\alpha = 0.5$ or 1.0. From eq 15, we can derive the bulge size along the contacting interface. The bulge size is equivalent to the spacing between bonding sites and is given as

$$2r = 2h\sqrt[14]{\left(\frac{\alpha k_B T}{\beta E h^3}\right)^3 \left(\frac{E}{p}\right)^5} \text{ with } \beta = \frac{1.2}{(1-\nu^2)^{3/4}} \quad (5)$$

To obtain the critical spacing $2r$, we need to find mechanical and structural properties of the cell membrane, including E and h in eq 5. The effective membrane thickness is about $h = 5$ nm.[19,20] Though there is no direct measurement of E, the bending stiffness of cell membrane is about $k \approx 20k_BT$.[19,20] Using the standard equation of bending stiffness

$$k = Eh^3/12(1-\nu^2) \quad (6)$$

we can get the in-plane modulus E of a cell membrane, which is about $E \approx 5-8$ MPa as ν changes from 0 to 0.5. Per our previous discussion, the repulsive bulge pressure p in eq 5 reflects the collective effect of all possible opposite forces to cell adhesion.[14,21] It is difficult to measure the resultant repulsive bulge pressure in the interface between a cell membrane and a ligand-coated substrate. Alternatively, we estimate the magnitude of the repulsive bulge pressure on the basis of the experimental measurement of the contractile stress in the focal adhesion region. From Dembo and Wang,[22] Balaban et al.,[23] Tan et al.,[24] and du Roure et al.,[25] the contractile stress σ in the focal adhesion region has an average of about $\sigma \approx 5.5$ kPa. Force balance in the bulge (Figure 1a) leads to an estimate of the bulge pressure p as

$$pA_b = \sigma A_c \quad (7)$$

where A_b is the effective area with an average bulge pressure p and A_c is the effective area of bound complexes around the bulge with contractile stress σ. By assuming that area A_c is of the same order of magnitude as A_b, we find that the bulge pressure p is on the order of σ. Equation 7 serves as a magnitude estimate of p in our dimensional analysis. The critical spacing is hence determined with known repulsive bulge pressure p and modulus E (derived from eq 6 using $h = 5$ nm and $k \approx 20k_BT$). Figure 2 shows the dependence of critical spacing on bulge pressure for two different Poisson ratios ν at $\alpha = 0.5$ and 1.0. For $p \approx 3 \times 10^3$ Pa, eq 5 leads to estimates of the critical spacing in the range of 39−48 nm. While assuming $p = 10^3$ Pa, we get the spacing in the range of 57−74 nm. An estimate of 71−89 nm is obtained if we take $p = 0.5 \times 10^3$ Pa. The agreement between the estimates based on our dimensional analysis and those obtained by experiments (58−73 nm[12]) is very good over a wide range of bulge pressure. In turn, this analysis gives us an estimate of the repulsive bulge pressure during the initiation of focal contact, which is on the order of 10^3 Pa.

Cell membranes are usually embedded with proteins, which will enhance the resistance to cell−membrane bending and effectively make the cell membrane thicker. Indeed, some groups reported $h \approx 10$ nm.[26,27] The spacing obtained using eq 5 for a fixed bending stiffness k actually has a very weak dependence on h. With eq 6 and $k \approx 20k_BT$, we can rewrite eq 5 as

$$2r = \sqrt[14]{\left(\frac{\alpha k_B T}{\beta E h^3}\right)^3 \frac{(Eh^3)^5}{p^5 h}} = c\sqrt[14]{\frac{k^5}{p^5 h}} \quad (8)$$

where c is a parameter that depends only on the Poisson ratio ν. A 10^4 -fold increase in h only doubles the spacing $2r$ for fixed k and p.

3. Conclusions

We have supplied a physical interpretation of the dependence of integrin-mediated cell adhesion on the spacing of ligand bonds in substrates. Our analysis is based on the assumption that the growth of focal adhesion is controlled by several major factors: bonding sites, thermal undulation, membrane deformation, and resultant repulsive bulge pressure in the contacting zone. Note that we have considered only the case in which large spacing between ligand sites—ligand starvation—gives rise to difficulty in focal adhesion formation. Alternatively, large spacing between receptors in cell membrane—receptor starvation—may also lead to unsuccessful focal adhesion growth. In most cases, receptors in membranes can diffuse quickly in cell membranes. The mean square distance of receptor proteins in cell membranes by diffusion is on the order of several to tens of nanometer per second.[28,29] We assume that receptors are abundant for focal adhesion growth in a time window of minutes to hours.[12] In contrast to the cases of ligand/receptor starvation, energy penalties of membrane deformation may also impose a minimum distance for integrin−ligand bonds. Again, because receptors in membranes can diffuse quickly and dissipate bending/stretching energy, the minimum length of integrin−ligand bonds should not hinder focal adhesion growth on a time scale of minutes to hours.

The results based on our analysis match quantitatively well with experiments.[12,30] In turn, on the basis of the experimental measurement of the critical spacing, we get an estimate of the repulsive bulge pressure near an adhesion patch during the initiation of focal contact, which is on the order of 10^3 Pa. However, we emphasize that there is consistency and inconsistency between the experiments[12,30] and our results. The consistency is that there is a critical spacing between ligand sites to ensure focal adhesion growth. The inconsistency lies in the points of view regarding governing mechanisms for the critical spacing of receptor−ligand bonds. Whereas our analysis indicates that physical forces may actually be the "molecular ruler" that governs receptor−ligand bond distance, Cavalcanti-Adam et al.[30] suggest that such critical spacing is controlled by a biochemical molecular ruler. More experiments are certainly desired to identify which molecular ruler—physical, biochemical, or a combination of both—governs the spacing of receptor−ligand bonds.

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