Theoretical study of the competition between cell-cell and cell-matrix adhesions

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Adhesions between neighboring cells or between cells and their surrounding tissue/matrix play a crucial role in a wide range of biological processes. In order to investigate the competitive mechanisms between cell-cell and cell-matrix adhesions, we here develop a theoretical framework for multiple interacting cells lying on a planar matrix coated with distributed ligands. This model allows us to study, from the viewpoints of energy and statistics, the effects of such physical mechanisms as binding energy of bonds, nonspecific interactions, elastic deformation of cell membranes, and mixing entropy. Our calculations show that cell-matrix adhesion cannot occur when the ligand density on the matrix is lower than a threshold value, and cell-cell adhesion does not happen for a high ligand density. Glycocalyx repulsion plays a more important role in cell-matrix adhesion than in cell-cell adhesion. In addition, it is found that the cell-cell adhesion density decreases as the number of cells increases.

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I. INTRODUCTION

Cells need to bind with other cells or an extracellular matrix in order to achieve many critical biological functions, e.g., development, blood clotting, lymphocyte homing, inflammatory, and immune responses. Adhesive interactions also provide an efficient pathway for cells to recognize their nearby cells or extracellular matrix and to detect mechanical stresses [1]. Indeed, cell-cell and cell-matrix adhesions take place simultaneously in a wide range of biological processes such as cell trafficking in and out of blood vessels, cell morphogenesis and migration, and malignant cell invasion [2]. Cell adhesion is mediated by a wide variety of cell adhesion molecules (CAMs) which interact with the molecules on the opposing cell or matrix. These adhesion molecules are termed as *receptors* and *ligands* [3] and their kinetic and mechanical properties have been studied by many researchers for their important roles in multifunctions of biological systems [4]. They can also be divided into two groups, homophilic and heterophilic. In the homophilic group, a CAM can bind with other CAMs of the same kind or, in other words, the receptors serve also as their own ligands, while in the heterophilic group, a CAM binds only with CAMs of other kinds. Homophilic adhesion normally takes place in the same type of cells, and heterophilic adhesion happens between different types of cells or between a cell and its surrounding tissue or matrix [1,4,5].

Cell adhesion is a dynamic process determined by the competition of strong short-range attraction forces within receptor-ligand pairs and long-range repulsion forces (e.g., undulation force) [6]. In addition, surface tension also affects the cellular adhesion process [7]. Considerable experimental and theoretical efforts have been directed toward understanding the biophysical mechanisms of cell-cell and cell-matrix adhesions. From the viewpoint of energy, cell adhesion is controlled by the minimization of the total free energy of the system, as a balance between competitive specific binding and nonspecific repulsion [7–9]. The synaptic patterns of two different lengths of bonds have been found to be important for *T* cells adhering to their target cells [10–12]. Dembo *et al.* [13,14] established a peeling model coupling the mechanical equations of an elastic membrane with the chemical kinetics associated with cell-matrix adhesion. Evans [15,16] proposed a continuum mechanics model of membrane-membrane adhesion accounting for the discrete kinetically trapped CAM cross-bridging forces. This model was later elaborated to examine the effects of some other factors that influence cell adhesion, e.g., the rigidity difference between adhesion and nonadhesion zones, and the nonuniform tension in the membrane [17]. Lipowsky *et al.* [18–21] established a statistical mechanics model to investigate the adhesion of multicomponent membranes mediated by specific adhesion molecules. Via Monte Carlo simulations, they studied the influences of several mechanisms, e.g., attractive binding energy of bonds, mixing entropy of molecules, and repulsive energy of glycocalyx, on the lateral phase separation of adhesion molecules. Weikl *et al.* [22,23] extended this model to examine the effects of two different types of bonds, and further demonstrated the influences of such mechanisms as nucleation diffusion and length mismatch on lateral phase separation. Evidently, a model system consisting of a ligand-containing vesicle, treated as a two-dimensional surface [24,25], and a receptor-coated substrate is an ideal and practical context to gain a deeper insight into some, though not all, fundamental aspects of cell adhesion [26–30]. The allocation functions of bound and free receptors in the vesicle can be determined from the equilibrium conditions of either energies or forces as a function of the adhesion area, the binding strength, and the receptor and ligand densities on the vesicle and the substrate [26,27]. Through a thermodynamic approach, Smith and Seifert [28] also discussed the influence of repelling molecules incorporated in the vesicle membrane on the formation of specific bonds.

In continuum mechanics models, the concentrations of diffusive receptors and ligands on the biomembrane and the

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matrix are described by continuous density functions of surface coordinates and time. However, continuum methods cannot account for the discrete features of cell-cell or cell-matrix interactions, nor can they describe the detailed evolutionary structures and compositions within the adhesion zones, which often play a significant role in the dynamic processes [17]. Therefore, statistical methods have been applied to consider the stochastic features of discrete molecular adhesions. In the statistical models [18–23,26–30], the surfaces of the biomembrane and the matrix are normally divided into a finite number of sites where receptors and ligands can occupy. Smith and Seifert [26] proposed a canonical formulation of statistical theory to study the effective adhesion strength of specifically bound vesicles. Based on their experimental observations that cell adhesion is sometimes mediated by a small number of receptor-ligand bonds, Zhu and co-workers [31–34] treated small-scale adhesions as a series of stochastic events. Zhu's kinetics model is especially efficient and has been widely applied for small systems, in which case cells do not compete for adhesion molecules [34].

Most previous theoretical efforts have been directed toward the adhesion between two cells or between a cell and a surrounding matrix. These well-defined models have captured, to different extents, a variety of crucial physical mechanisms in cell adhesion and some relevant biological processes. In many realistic and experimental situations, a cell adheres to other cells and a matrix simultaneously. The properties of a matrix usually have a significant influence on the adhesion behavior of cells lying on it, as has been demonstrated by experiments [2,35]. Hegedüs *et al.* [2] examined the interplay of cell-cell and cell-matrix interactions and its role in the malignant cell invasion process. Furthermore, tissue formation and maintenance are regulated by different mechanical signals between cell-cell and cell-matrix interactions [35]. The immigration of interacting or aggregated cells is also sensitive to the relative strength of mechanical signals from the surrounding matrix, besides those from neighboring cells. Cell-cell adhesion competes for CAMs directly with cell-matrix adhesion, and this affects the adhesion strength between cells and the extracellular matrix. Thus, of importance is to study the interplay between cell-cell and cell-matrix adhesions.

Therefore, the present paper aims to theoretically study the adhesion of two or more cells with mobile receptors adhering to a matrix coated with immobilized ligands. We account for the discrete thermodynamic process by using the classical Bell's adhesion model [8] in combination with Smith's canonical formulation for specific cell-matrix adhesion [26–28]. Cell-cell and cell-matrix adhesions take place simultaneously and compete for the adhesion molecules because their number is limited. Based on the considerations of biophysical mechanisms of cell adhesion, we will study the adhesion problems of two, three, and an infinite number of cells positioned abreast on a matrix and discuss the competitive relation between cell-cell and cell-matrix adhesions. The functional of the total free energy of the system is first formulated, and a Monte Carlo method is utilized to find the equilibrium state that minimizes the free energy. The influences of such factors as the ligand density on the matrix, intrinsic properties (equilibrium lengths and spring constants) of cell adhesion molecules, and surface tension are investigated.

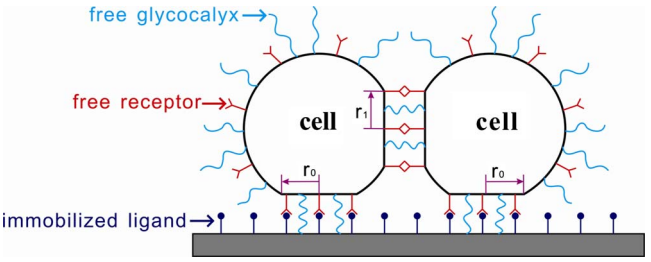

FIG. 1. (Color online) Two contacting cells lying on an extracellular matrix coated with uniformly distributed ligands. The ligands on the matrix are fixed, while the receptors and glycocalyx molecules on cell membranes are freely mobile. The contact radii of cell-matrix and cell-cell adhesion zones are r_0 and r_1, respectively.

II. ADHESION MODEL

To study the competitive biophysical mechanisms of cell-cell and cell-matrix adhesions that happen simultaneously, we first propose a statistical mechanics model based on Bell's adhesion model [8] in combination with the canonical formulation in Refs. [26–28]. For the sake of simplicity, we first consider only two cells adhering to a planar extracellular matrix, as shown in Fig. 1, and the model will be extended to the cases of three or an infinite number of cells in Sec. V. Evidently, cell-cell and cell-matrix adhesions are elicited by different intracellular signaling pathways. The CAMs on the cell membranes can mobile freely, and then they can form bonds at any position in the cell-cell adhesion zone. By contrast, the CAMs on the matrix are usually fixed and immobile and, hence, the receptors on the cell membranes cannot bind to the matrix until they move to the seats with immobile ligands on the matrix surface. On one hand, therefore, cell-matrix adhesion is dominantly reliant on the ligand concentration on the matrix, as observed in experiments [36,37]. On the other hand, the two processes of cell-cell and cell-matrix adhesions compete with each other due to the limited number of CAMs on the cell membranes.

In our current study, we assume that the immobilized ligands on the matrix are uniformly distributed, while the receptors on the cell membranes can move freely. The glycocalyx, which acts as repellers [20,21,28], is also freely mobile on the cells. The two cells are assumed to be of the same type and have the same volume, area, and receptor density. Therefore, the bonds formed during cell-cell adhesion are receptor-receptor or homophilic pairs, while those between the cells and the matrix are receptor-ligand or heterophilic pairs. The receptor-ligand interaction between a cell and the matrix can take place only within their contact zone A_0, and the receptors on the two cells interact only within their contact zone A_1 (see Fig. 1).

The total Gibbs free energy G of the cell-matrix system can be written as

$$G = U + \Gamma + W - TS, \tag{1}$$

where U is the total binding energy of all the receptor-receptor and receptor-ligand bonds, Γ is the nonspecific in-

teraction energy associated with the long-range force, W is the elastic deformation energy of the cell membranes, T is the absolute temperature, and S is the mixing entropy.

A. Binding energy of bonds

Forming a single bond, a receptor-receptor or a receptor-ligand pair, will release a certain amount of free energy, E_a, referred to as the binding energy. It takes the form [26]

$$E_a = C_b k_B T, \tag{2}$$

where k_B is the Boltzmann constant and C_b is a constant in the range 5–20 for different bonds [38]. The total binding energy of the system is the sum of all the bonds formed in the three contact zones, and it serves as the main driving force for the cell-cell and cell-matrix adhesions. The total free energy change of the system due to binding is expressed as

$$U = -2N_{b0}E_a - N_{b1}E_a, \tag{3}$$

where N_{b0} and N_{b1} are the numbers of bonds formed in each cell-matrix contact zone and the cell-cell contact zone, respectively.

B. Nonspecific interaction energy

Such forces as electrostatic force, arising from the negative charges on the cell surfaces, and steric stabilization force, arising from the glycocalyx molecules coated on the cell membranes, tend to reject cell adhesion [8]. If the binding strengths of bonds are not sufficiently strong, cell adhesion will not take place due to the long-range repulsive forces. According to Burroughs and Wülfing [12], who studied the driving force of synapse dynamics, the long-range interaction potential around the balance distance L_b can be written in the following simplified form

$$\Gamma(h) = \frac{1}{2}\xi(h - L_b)^2, \tag{4}$$

where the coefficient ξ represents the strength of the glycocalyx potential, and h is the distance between the membrane surface and the matrix. This potential was also adopted and discussed in Refs. [39,40]. Here, we modify the above equation to the more complicated situation of current interest. It is reasonable to assume that the glycocalyx on the cell membranes can move freely and the parameter ξ has different values in the cell-cell and cell-matrix adhesion zones. For the glycocalyx, its equilibrium length L_g is taken as the balance distance, and its spring constant ξ_g as the strength of glycocalyx potential. For receptor-ligand and receptor-receptor pairs, we also take their equilibrium lengths L_{b0} and L_{b1} as their balance distances, and their spring constants ξ_{b0} and ξ_{b1} as their strengths of the corresponding potentials, respectively. Therefore, we use Eq. (4) to describe the interaction for each kind of molecular pairs. This potential can reduce to the single-parameter model for the interaction of molecules between two surfaces adopted in Refs. [26,28,40]. In the present paper, the three-parameter model in Eq. (4) is assumed in order to examine the influences of such factors as the mismatch between the equilibrium lengths of bonds and glycocalyx. In addition, it is also noticed that we neglect the interaction potential outside the adhesion zones or, in other words, the potential function has been truncated.

The interaction potential energy of the whole system, which is the sum of all molecules in adhesion zones, can be written as

$$\Gamma = N_{b0}\xi_{b0}(h_0 - L_{b0})^2 + \frac{1}{2}N_{b1}\xi_{b1}(h_1 - L_{b1})^2 + N_{g0}\xi_g(h_0 - L_g)^2 + N_{g1}\xi_g(h_1 - L_g)^2, \tag{5}$$

where the subscripts 0 and 1 stand for the parameters in the cell-matrix contact zone A_0 and cell-cell contact zone A_1, respectively, N_{g0} and N_{g1} are the numbers of glycocalyx molecules, and h_0 and h_1 are the separation distances of the opposing surfaces in the corresponding zones. From $\partial\Gamma/\partial h_i = 0$ ($i=0$ or 1), it is known that the equilibrium separation h_i is just between the two balance distances of bonds and glycocalyx, that is, $L_{bi} \le h_i \le L_g$. This indicates that the glycocalyx plays a repulsive role in the adhesions while the receptor-receptor and receptor-ligand bonds are in the attractive range.

C. Elastic deformation energy of cell membranes

During the progress of cell adhesion, cells change their shape and increase the elastic energy of their membranes to oppose the adhesive contact. Exact determination of cell shapes in adhesion is an important but complicated issue [18], especially for the current problem consisting of multiple cells adhering to a matrix. To focus our attention on the competitive mechanisms between cell-cell and cell-matrix adhesions, we assume each cell has a truncated sphere shape. Denote the original radius of the spherical cell as R_0 and the original surface area of each cell $A_t = 4\pi R_0^2$. The volume of the truncated sphere of one cell illustrated in Fig. 1 is

$$V = \frac{4}{3}\pi R^3 - \frac{1}{3}\pi h_0^2(3R - h_0) - \frac{1}{3}\pi h_1^2(3R - h_1), \tag{6}$$

where $h_0 = R - \sqrt{R^2 - r_0^2}$, $h_1 = R - \sqrt{R^2 - r_1^2}$, r_0, and r_1 are the contact radii of the adhesion zones A_0 and A_1, respectively, and R is the radius of the deformed sphere. It is generally reasonable to assume that the volume V keeps a constant during cell deformation [41,42], that is, $V = 4\pi R_0^3/3$. Thereby, R is approximately expressed as

$$\frac{R}{R_0} = 1 + \frac{1}{16}\left(\frac{r_0^4}{R_0^4} + \frac{r_1^4}{R_0^4}\right). \tag{7}$$

Then the surface area change of each cell is

$$\Delta A = 2\pi R\sqrt{R^2 - r_0^2} + 2\pi R\sqrt{R^2 - r_1^2} + A_0 + A_1 - A_t. \tag{8}$$

The increment of the elastic energy of cell membranes is the sum of bending and stretching components [43]

$$\Delta W = \frac{1}{2}Bk_B T(\kappa_1 + \kappa_2 - \kappa_0)^2(2A_0 + A_1) + 2\gamma\Delta A, \tag{9}$$

where Bk_BT is the bending stiffness, κ_1 and κ_2 are theprincipal curvatures, κ_0 is the spontaneous curvature, γ represents the surface tension of cell membrane, and ΔA is the deformation-induced surface area change of each cell. In Eq. (9), the two terms are the bending energy and the stretching energy, respectively. Compared with the binding energy of bonds on the order of about 10^{-17} J/μm^2, the bending energy of cells is typically on the order of about 10^{-21} J/μm^2 and then can be neglected [26]. Therefore, we simplify Eq. (9) as

$$\Delta W = 2\gamma\Delta A. \tag{10}$$

If there is no cell-matrix adhesion but merely cell-cell adhesion, Eqs. (8) and (10) will degenerate to the expression for a single cell adhering to a matrix given by Coombs *et al.* [7].

D. Mixing entropy

The mixing entropy S can be calculated by counting the number Ω of all possible conformations of the positions of all molecules in the whole system. One has

$$\Omega = \Omega_1 \times \Omega_2, \tag{11}$$

where Ω_1 is the number of possible conformations of the positions of molecules in the system except in the cell-cell adhesion zone, and Ω_2 is the number of possible conformations of the positions of molecules in the cell-cell adhesion zone.

Let N_t denote the total number of receptors in each cell, N_{f0} and N_{f1} the numbers of free receptors within A_0 and A_1, respectively, ρ_0 the fraction of the ligand-occupied surface area of the matrix, also referred to as the ligand density, and α the gyration area of a single receptor. For simplicity, each ligand on the matrix and each repelling molecule, glycocalyx, are assumed to have the same gyration area with the receptor.

In the calculation of Ω_1, one can place $N_t - N_{b0} - N_{f0} - N_{b1} - N_{f1}$ free receptors on the total available sites in the free or nonadhesive zone of each cell, N_{f0} free receptors on the available sites not occupied by ligands in A_0 of each cell, N_{b0} receptor-ligand pairs on the total available ligand sites in A_0 of each cell, N_{g0} repellers on the available sites not occupied by ligands and free receptors in A_0 of each cell, and $N_g - N_{g0} - N_{g1}$ repellers on the total available sites not occupied by receptors in the free part of each cell. Then we get

$$\Omega_1 = \begin{pmatrix} \frac{1}{\alpha}(A_t + \Delta A - A_0 - A_1) \\ N_t - N_{b0} - N_{f0} - N_{b1} - N_{f1} \end{pmatrix}^2 \begin{pmatrix} \frac{1}{\alpha}(1-\rho_0)A_0 \\ N_{f0} \end{pmatrix}^2 \begin{pmatrix} \frac{1}{\alpha}\rho_0 A_0 \\ N_{b0} \end{pmatrix}^2 \begin{pmatrix} \frac{1}{\alpha}(1-\rho_0)A_0 - N_{f0} \\ N_{g0} \end{pmatrix}^2 \times \begin{pmatrix} \frac{1}{\alpha}(A_t + \Delta A - A_0 - A_1) - (N_t - N_{b0} - N_{f0} - N_{b1} - N_{f1}) \\ N_g - N_{g0} - N_{g1} \end{pmatrix}^2. \tag{12}$$

Similarly, in the calculation of Ω_2, one can place N_{f1} free receptors on available sites in A_1 of each cell, N_{b1} receptor-receptor pairs on the available sites not occupied by free receptors in A_1, and N_{g1} repellers on the available sites not occupied by free receptors and bonds in A_1 of each cell. Therefore, Ω_2 reads

$$\Omega_2 = \begin{pmatrix} \frac{1}{\alpha}A_1 \\ N_{f1} \end{pmatrix} \begin{pmatrix} \frac{1}{\alpha}A_1 - N_{f1} \\ N_{f1} \end{pmatrix} \begin{pmatrix} \frac{1}{\alpha}A_1 - 2N_{f1} \\ N_{b1} \end{pmatrix} \times \begin{pmatrix} \frac{1}{\alpha}A_1 - 2N_{f1} - N_{b1} \\ N_{g1} \end{pmatrix} \begin{pmatrix} \frac{1}{\alpha}A_1 - 2N_{f1} - N_{b1} - N_{g1} \\ N_{g1} \end{pmatrix}. \tag{13}$$

Treating the mobile bonds and other free molecules as solutes in a two-dimensional solvent along the interface, the mixing entropy can be written as [26,44,45]

$$S = k_B \ln \Omega. \tag{14}$$

Then, S can be calculated by using the Stirling formula for the factorials of large numbers, but its lengthy expression is not given here. If there exists no cell-cell adhesion but only cell-matrix adhesion, Eqs. (11)–(14) will reduce to the expressions for a single cell derived by Smith and Seifert [26].

III. COMPUTATIONAL PROCEDURE

The adhesion model presented in the preceding section can account for the effects of binding energy, nonspecific interactions, membrane deformation, and mixing entropy. Due to the intrinsic complexity in the coupled cell-cell and cell-matrix adhesions, a Monte Carlo numerical method is here adopted to study the interplaying mechanisms of cell-cell and cell-matrix adhesions. We seek the equilibrium state by minimizing the Gibbs free energy of the system. For convenience, we use the following normalized or dimensionless parameters in the simulations:

$$g = \frac{G}{N_t k_B T}, \quad Q_0 = \frac{\xi_{b0}}{\xi_g}, \quad Q_1 = \frac{\xi_{b1}}{\xi_g}, \quad \rho_{cm} = \frac{\alpha N_{b0}}{A_0},$$

$$\rho_{cc} = \frac{\alpha N_{b1}}{A_1}$$

$$a_t = \frac{A_t}{\alpha N_t}, \quad \Delta a = \frac{\Delta A}{\alpha N_t}, \quad a_0 = \frac{A_0}{\alpha N_t}, \quad a_1 = \frac{A_1}{\alpha N_t}, \quad \gamma' = \frac{\gamma\alpha}{k_B T},$$

$$n_{b0} = \frac{N_{b0}}{N_t}, \quad n_{b1} = \frac{N_{b1}}{N_t}, \quad n_{f0} = \frac{N_{f0}}{N_t}, \quad n_{f1} = \frac{N_{f1}}{N_t}, \quad (15)$$

$$n_{g0} = \frac{N_{g0}}{N_t}, \quad n_{g1} = \frac{N_{g1}}{N_t}.$$

The dimensionless adhesion areas (a_0, a_1), the normalized numbers (n_{b0}, n_{b1}) of bonds, the normalized numbers (n_{f0}, n_{f1}) of free receptors, the dimensionless numbers (n_{g0}, n_{g1}) of repellers within the adhesion zones, and the separation distances (h_0, h_1) are varied in certain ranges in order to minimize the dimensionless energy function g. The ten variables must satisfy the following constraint conditions:

(i) Evidently, the dimensionless adhesion areas and separation distances cannot be negative. Besides, the contact radii (r_0, r_1) cannot exceed the original radius R_0 of the cell [7]. Hence, one has

$$a_0 \geq 0, \quad a_1 \geq 0, \quad h_0 \geq 0, \quad h_1 \geq 0,$$

$$a_0 \leq a_t/2, \quad a_1 \leq a_t/2. \quad (16)$$

(ii) Since the numbers of receptors (bound or free) in the adhesion zones cannot exceed the total number of receptors on the cell membranes, the parameters n_{b0}, n_{b1}, n_{f0}, and n_{f1} should satisfy the inequalities

$$0 \leq n_{b0} \leq 1, \quad 0 \leq n_{b1} \leq 1, \quad 0 \leq n_{f0} \leq 1,$$

$$0 \leq n_{f1} \leq 1, \quad 0 \leq n_{b0} + n_{b1} + n_{f0} + n_{f1} \leq 1. \quad (17)$$

(iii) The numbers of repelling molecules must fulfill the following conditions:

$$0 \leq n_{g0} \leq n_g, \quad 0 \leq n_{g1} \leq n_g, \quad 0 \leq n_{g0} + n_{g1} \leq n_g. \quad (18)$$

(iv) The number of bonds in A_0 cannot exceed the total number of ligands in the same region, the number of free molecules in A_0 is restricted by the number of the available sites in A_0 that are not occupied by ligands, and the number of all molecules (bonds, free receptors, and repellers) in A_1 cannot exceed the number of all sites in A_1. Therefore, it is required that

$$n_{b0} \leq \rho_0 a_0, \quad n_{f0} + n_{g0} \leq (1 - \rho_0) a_0, \quad (19)$$

$$2n_{f1} + n_{b1} + 2n_{g1} \leq a_1.$$

Thus the problem becomes the minimization of the free energy g as a function of the ten variables subjected to the 17 constraint conditions in Eqs. (16)–(19). These variables are also denoted as x_i for simplicity of calculations. Due to the larger numbers of variables and constraints, it is difficult to get the minimum values of g using conventional numerical methods. Here, we develop a Monte Carlo numerical method to solve this complicated problem of nonlinear mathematical programming. In each step, we specify a random variation Δx_i to a random variable x_i and calculate the corresponding change Δg of the free energy, which is adopted as the accept/reject criterion to judge whether the trial perturbation will be accepted or not. If $\Delta g < 0$, we accept this variation Δx_i, and update x_i by $x_i + \Delta x_i$ in the next step; otherwise we will still use x_i in the next step. In this manner, all the ten variables will gradually approach their optimal values, corresponding to the minimum of the free energy function. The solution algorithm is briefly described as follows:

(i) Create the initial random values of the ten variables, under all the 17 constraint conditions in Eqs. (16)–(19).

(ii) Generate a random integer i, in the range of $1 \leq i \leq 10$, such that the variable x_i will be chosen to change in the following step.

(iii) Generate a random infinitesimal variation Δx_i.

(iv) Replace x_i with $x_i + \Delta x_i$ and check whether the ten variables satisfy the 17 constraint conditions or not. If they are all fulfilled, then go to step (v); otherwise, return to (ii).

(v) Calculate the corresponding variation of the free energy, $\Delta g = (\Delta U + \Delta\Gamma + \Delta W - T\Delta S)/(N_t k_B T)$. If $\Delta g < 0$, we will replace x_i with $x_i + \Delta x_i$ in the next step; otherwise, we will still use x_i.

(vi) Repeat steps (ii)–(v) until the minimum value of g is achieved.

The fast convergency, high efficiency, and good accuracy of this method have been demonstrated by a large number of calculation examples, some of which will be given in Sec. IV.

IV. RESULTS AND DISCUSSIONS

In this section, we will study the influences of such factors as the ligand density, the bond properties of receptor-receptor and receptor-ligand pairs, and the resistance mechanisms on the competition between cell-cell and cell-matrix adhesions. As aforementioned, the resistances result mainly from the membrane elasticity and the glycocalyx repulsive potential. In the examples, we assume that each cell has a surface area of 500 μm^2 and contains 10^5 receptors [8] and 5×10^6 glycocalyx molecules [9] on its surface. The receptor-ligand and receptor-receptor pairs are assumed to have the same equilibrium lengths of $L_{b0} = L_{b1} = 11$ nm and the same spring constants of $\xi_{b0} = \xi_{b1} = 0.25$ pN/nm [45]. The equilibrium length of glycocalyx is set to be $L_g = 30$ nm, and the corresponding spring constant is $\xi_g = 0.01$ pN/nm [45]. Additionally, the gyration area of a single molecule α and C_b are taken as 38.5 nm^2 and 10, respectively [26], and the surface tension is $\gamma = 0.1$ pN/nm [7]. Unless stated otherwise, these values will be used in the calculations throughout the paper.

A. Effect of the ligand density on the matrix

We first examine the role of the ligand density ρ_0 on the matrix in the competitive process of cell-cell and cell-matrix adhesions, as shown in Fig. 2. It is found that when the

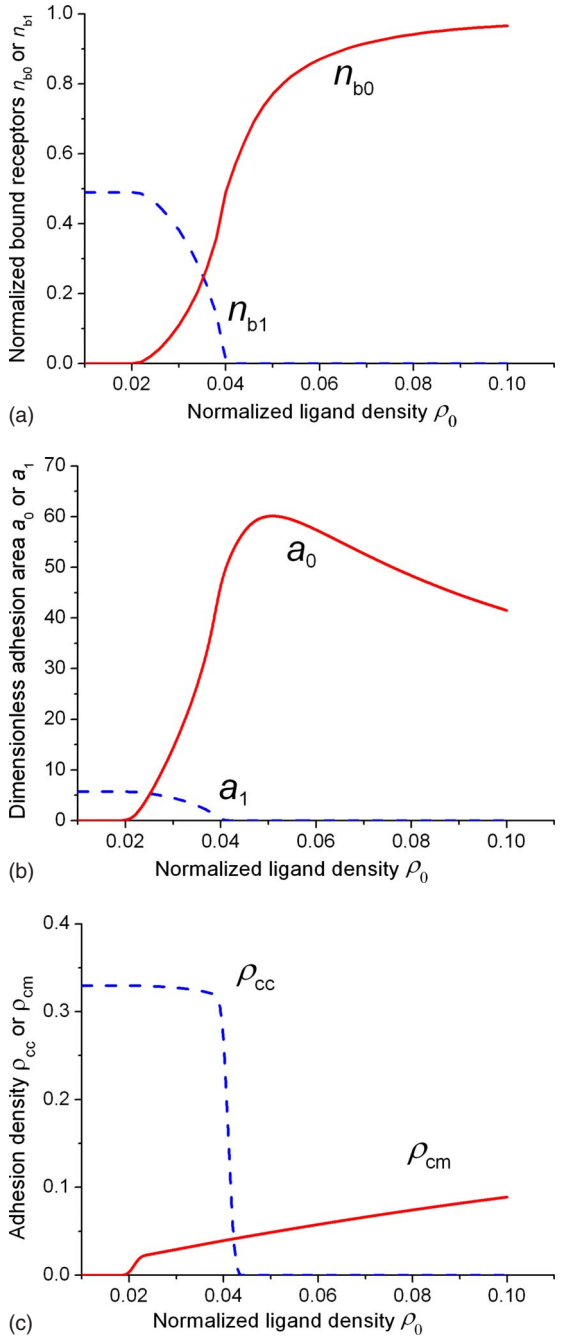

FIG. 2. (Color online) Effects of the ligand density ρ_0 on the competitive cell-cell and cell-matrix adhesions. The variations of (a) normalized bound receptors n_{b0} and n_{b1}, (b) dimensionless adhesion areas a_0 and a_1, and (c) adhesion densities ρ_{cm} and ρ_{cc}.

normalized ligand density ρ_0 is lower than a threshold value of about 0.022, the normalized bound receptor number n_{b0} and the normalized cell-matrix adhesion area a_0 remain almost zero [Figs. 2(a) and 2(b)], indicating that the two cells will adhere to each other but not to the matrix. Due to the low concentration and immobility of ligands on the matrix, only a small number of receptor-ligand pairs can form between the cells and the matrix. In this case, the matrix cannot provide sufficient positions to form bonds with the receptors on the cell surfaces, and the binding energy released due to cell-matrix adhesion is insufficient to overcome the resistances resulting from surface tension and glycocalyx repulsion. With the increase in ρ_0 from 0 to 0.022, the fraction n_{b0} of the bound receptors in the cell-matrix adhesion zone and the normalized area a_0 of the cell-matrix adhesion zone remain almost zero. At the same time, the fraction n_{b1} of the bound receptors in the cell-cell adhesion zone and the normalized area a_1 of the cell-cell adhesion zone keep constant, as can be seen from Fig. 2. Therefore, there exists a critical ligand density below which cells will not adhere to the matrix, as is consistent with experimental observations [36,37].

With the increase in ρ_0 in the range of 0.022–0.04, the matrix surface will have more and more sites where the receptors can bind the ligands, leading to an increase in the binding energy per unit area. Therefore, the normalized bound receptor number n_{b0} increases, whereas the normalized bound receptor number n_{b1} decreases as a result of the conservation of the total number of receptors. The normalized cell-matrix adhesion area a_0 and a_1 have the same changing tendencies with n_{b0} and n_{b1}, respectively. When $\rho_0 > 0.04$, the two cells will adhere only to the matrix but not to each other. In other words, there exists a critical ligand density above which cell-cell adhesion will not happen.

To more clearly illustrate this competitive mechanism, define the cell-cell adhesion density $\rho_{cc}=\alpha N_{b1}/A_1$ and the cell-matrix adhesion density $\rho_{cm}=\alpha N_{b0}/A_0$ to represent the corresponding binding energy values per unit area in A_1 and A_0, respectively. It is interesting to find from Fig. 2(c) that ρ_{cc} is much larger than ρ_{cm} when both cell-cell and cell-matrix adhesions develop. In other words, the bond density in A_1 is always much higher than that in A_0 provided that cell-cell adhesion exists. This observation can be understood from the different mobility properties of bonds in the contact zones A_0 and A_1. The receptor-ligand pairs can form only on those positions with immobile ligands in the cell-matrix adhesion zone. Therefore, the adhesion density ρ_{cm} is limited by the constant ligand density ρ_0 in A_0. In contrary, the receptor-receptor pairs can appear on any positions in the cell-cell adhesion zone, and the adhesion density ρ_{cc} in A_1 can reach a much higher value. A similar conclusion was recently drawn by Smith *et al.* [29], who compared the results of cell-matrix adhesions with mobile or immobile integrin molecules.

The minimization of the free energy requires that n_{b0} satisfies the condition $\partial g/\partial n_{b0}=0$. Thereby, we derive

$$-C_b+\frac{Q_0\xi_g}{2k_BT}(h_0-L_{b0})^2+\ln\frac{a_0(1-\rho_0)-(n_{f0}+n_{b0})}{n_{f0}}+\ln\frac{n_{b0}}{\rho_0 a_0-n_{b0}}=0, \tag{20}$$

from which the cell-matrix adhesion density ρ_{cm} is solved as

$$\rho_{cm}=\frac{n_{b0}}{a_0}=\rho_0\left\{\exp\left[-C_b+\frac{Q_0\xi_g}{2k_BT}(h_0-L_{b0})^2\right]+1-\frac{n_{f0}}{a_0(1-\rho_0)-(n_{f0}+n_{b0})}\right\}^{-1}. \tag{21}$$

In most situations, n_{f0} is much smaller than $a_0(1-\rho_0)-(n_{f0}+n_{b0})$ and h_0 is nearly constant. Therefore, ρ_{cm} is approxi-

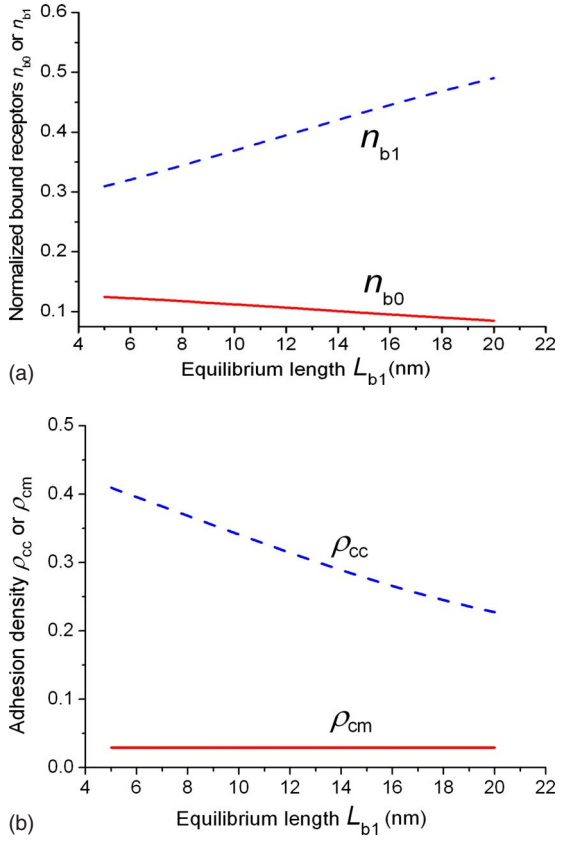

FIG. 3. (Color online) Influences of the equilibrium length L_{b1} of receptor-receptor pairs on the competitive cell-cell and cell-matrix adhesions, where we take $\rho_0=0.03$. The variations of (a) n_{b0} and n_{b1} and (b) ρ_{cm} and ρ_{cc}.

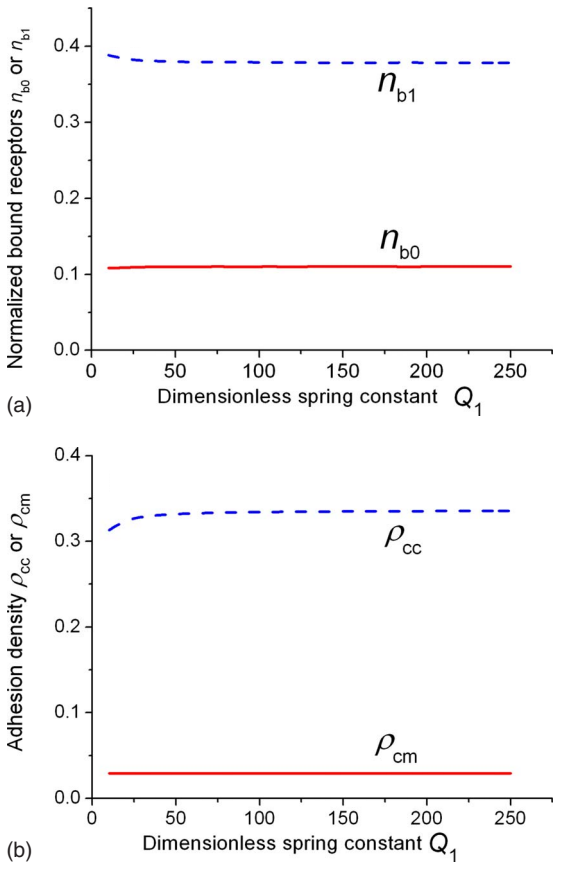

FIG. 4. (Color online) Influences of the dimensionless spring constants Q_1 of receptor-receptor pairs on the competitive cell-cell and cell-matrix adhesions, where we set $\rho_0=0.03$. The variations of (a) n_{b0} and n_{b1} and (b) ρ_{cm} and ρ_{cc}.

mately proportional to the ligand density ρ_0, as shown in Fig. 2(c).

B. Effect of bond properties of receptor-receptor and receptor-ligand pairs

Now we vary the equilibrium lengths and spring constants of receptor-receptor and receptor-ligand pairs in order to explore their influences on the equilibrium state of the system. The variations of the allocation functions (n_{b0}, n_{b1}) of bound receptors and the adhesion densities (ρ_{cm}, ρ_{cc}) are shown in Fig. 3 with respect to the equilibrium length L_{b1} of receptor-receptor pairs. As the value of L_{b1} increases, more and more receptors (n_{b1}) will move into the cell-matrix adhesion zones, and correspondingly the cell-cell adhesion density ρ_{cc} decreases. This is because that the nonspecific interaction originating from the bonds and repellers becomes less and less significant with increasing L_{b1}.

The minimization of the free energy with respect to the distance h_1 between two cells requires that $\partial g/\partial h_1=0$. Thereby, we obtain

$$h_1 = \frac{Q_1 L_{b1} n_{b1} + 2L_g n_{g1}}{Q_1 n_{b1} + 2n_{g1}}. \tag{22}$$

The corresponding potential energy is

$$\Gamma_1 = \xi_g (h_1 - L_g)^2 n_{g1} + \frac{1}{2}\xi_{b1}(h_1 - L_{b1})^2 n_{b1}. \tag{23}$$

Substituting Eq. (22) into Eq. (23) yields

$$\Gamma_1 = \frac{Q_1 n_{b1} n_{g1}}{Q_1 n_{b1} + 2n_{g1}} \xi_g (L_g - L_{b1})^2. \tag{24}$$

This equation shows that as L_{b1} increases toward L_g, the resistance Γ_1 to the cell-cell adhesion trails off and, hence, the two cells tend to adhere to each other, as is consistent with the results in Fig. 3.

With the increase in the dimensionless spring constants Q_1 of receptor-receptor pairs, the variations of (n_{b0}, n_{b1}) and (ρ_{cm}, ρ_{cc}) are shown in Fig. 4. Their changes are relatively slow, demonstrating that in comparison with the parameter L_{b1}, the parameter Q_1 has a weaker influence on the competitive process. It is known from Eq. (24) that Γ_1 increases with increasing Q_1, since $\partial\Gamma_1/\partial Q_1 > 0$. Therefore, the cell-cell adhesion density ρ_{cc} will increase and provide more binding energy to overcome the increasing potential Γ_1. Additionally, the coefficient $Q_1 n_{b1} n_{g1}/(Q_1 n_{b1} + 2n_{g1})$ in Eq. (24) changes little with Q_1, especially when Q_1 is large. Thus, the cell-cell adhesion shows a relatively weak dependence on Q_1, as is consistent with Fig. 4.

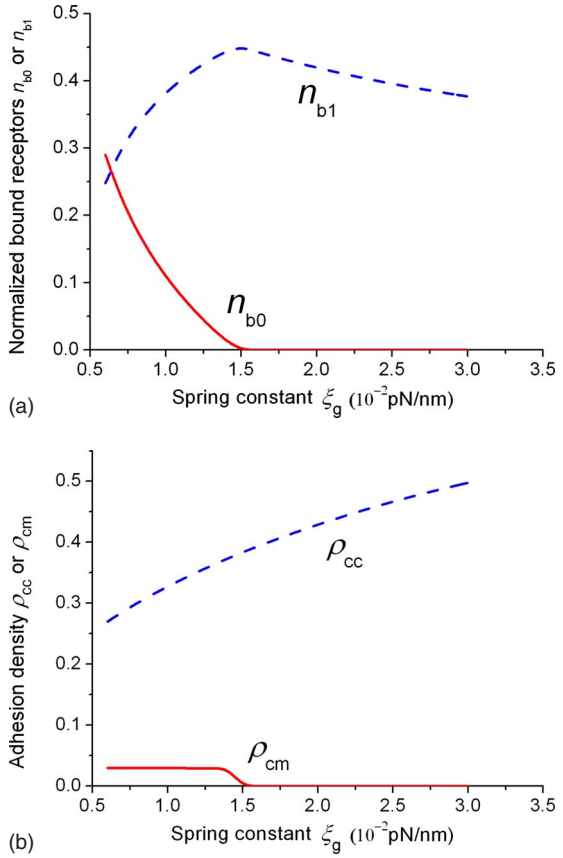

FIG. 5. (Color online) Effects of the spring constant of repellers ξ_g, where we take $\rho_0=0.03$. The variations of (a) n_{b0} and n_{b1} and (b) ρ_{cm} and ρ_{cc}.

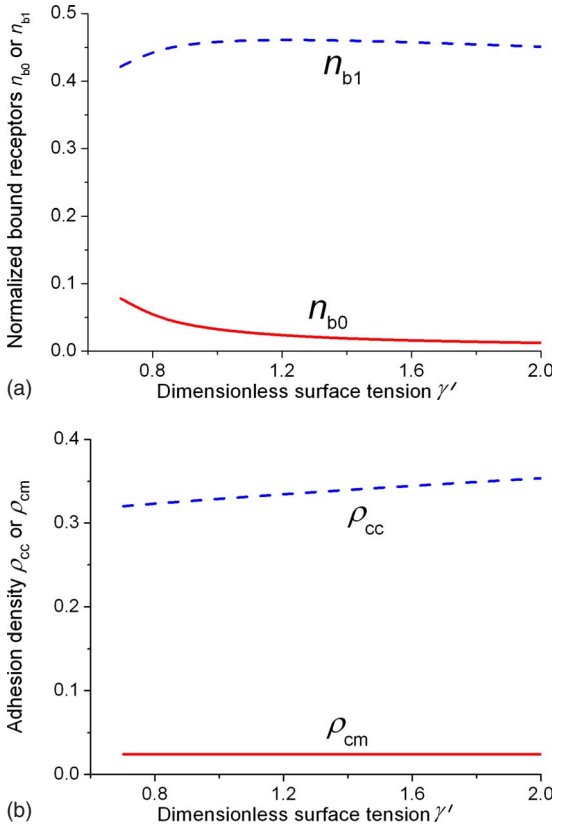

FIG. 6. (Color online) Effects of the dimensionless surface tension γ', where we take $\rho_0=0.025$. The variations of (a) n_{b0} and n_{b1} and (b) ρ_{cm} and ρ_{cc}.

C. Effect of adhesion resistance

We further examine the effect of resistance forces on the adhesions by varying the spring constant ξ_g of repellers and the surface tension γ of the cell membrane. As the resistance force ξ_g enhances from 0.006 to 0.015 pN/nm, the normalized bound receptor number n_{b0} rapidly decreases from about 0.3 to 0, as shown in Fig. 5(a). Correspondingly, the fraction n_{b1} of bound receptors in the cell-matrix adhesion zone A_1 increases because more and more receptors are unleashed from the zone A_0 and they can bind the ligands on the matrix. When ξ_g is larger than the threshold value $\sim$0.015 pN/nm, the cells will not adhere to the matrix for the enhanced repulsive strength of glycocalyx potential. This observation demonstrates that glycocalyx repulsion plays a more significant role in cell-matrix adhesion than in cell-cell adhesion. This is because that in A_0, the adhesion density is limited by the number density of immobile ligands on the matrix and the receptor-ligand binding energy cannot reach a higher level to overcome a strong glycocalyx repulsion, while in A_1, a larger adhesion density can be achieved through the mobility of receptors. Due to the constant density and immobility of ligands on the matrix surface, the cell-matrix adhesion density ρ_{cm} keeps a constant of about 0.028 in the range of $\xi_g<0.015$ pN/nm, as shown in Fig. 5(b). This constant of ρ_{cm} is very close to the specified value of $\rho_0=0.03$, implying that almost all ligands in the adhesion zone A_0 have bound the receptors on the cell surfaces. In addition, it is noticed that the cell-cell adhesion density ρ_{cc} increases with the increase in the spring constant ξ_g of repellers, and this strengthening of adhesion for large repulsive strength was also observed by Smith and Seifert [28].

Figure 6 illustrate the influence of the dimensionless surface tension γ'. Evidently, the surface tension of cell membranes shows a relatively weaker influence on the adhesion process than the glycocalyx potential. With the increase of γ' in the considered range, cell-matrix adhesion always happens in spite of the slight decrease of n_{b0}, and ρ_{cm} keeps a constant of about 0.024, which is very close to the specified ligand density ρ_0 of 0.025.

V. MODEL FOR THREE OR AN INFINITE NUMBER OF CELLS

The above presented model can be extended to more complicated cases. In what follows, we will reformulate this model for two other situations, namely, three and an infinite number of cells or vesicles positioned abreast on a matrix coated with distributed ligands. It is worth mentioning that an array of vesicles may be easier to reach a thermodynamic equilibrium than an array of cells because of the complicated biological behaviors of cells.

A. Case of three cells

In the case of three cells positioned abreast on the matrix, the binding energy U, the nonspecific interaction energy Γ,

the elastic deformation energy of cell membranes W, and the mixing entropy S are rewritten as

$$U = -3N_{b0}E_a - 2N_{b1}E_a, \tag{25}$$

$$\Gamma = \frac{3}{2}N_{b0}\xi_{b0}(h_0 - L_{b0})^2 + N_{b1}\xi_{b1}(h_1 - L_{b1})^2 + \frac{3}{2}N_{g0}\xi_g(h_0 - L_g)^2 + \frac{3}{2}N_{g1}\xi_g(h_1 - L_g)^2, \tag{26}$$

$$\Delta W = \gamma \Delta A_t, \tag{27}$$

$$S = \ln(\Omega_1 \times \Omega_2), \tag{28}$$

where ΔA_t is the total variation of surface areas of the three cells, and

$$\Omega_1 = \begin{pmatrix} \frac{1}{\alpha}(A_t + \Delta A - A_0 - A_1) \\ N_t - N_{b0} - N_{f0} - N_{b1} - N_{f1} \end{pmatrix}^3 \begin{pmatrix} \frac{1}{\alpha}(1-\rho_0)A_0 \\ N_{f0} \end{pmatrix}^3 \begin{pmatrix} \frac{1}{\alpha}\rho_0 A_0 \\ N_{b0} \end{pmatrix}^3 \begin{pmatrix} \frac{1}{\alpha}(1-\rho_0)A_0 - N_{f0} \\ N_{g0} \end{pmatrix}^3 \times \begin{pmatrix} \frac{1}{\alpha}(A_t + \Delta A - A_0 - A_1) - (N_t - N_{b0} - N_{f0} - N_{b1} - N_{f1}) \\ N_g - N_{g0} - N_{g1} \end{pmatrix}^3, \tag{29}$$

$$\Omega_2 = \begin{pmatrix} \frac{1}{\alpha}A_1 \\ N_{f1} \end{pmatrix}^2 \begin{pmatrix} \frac{1}{\alpha}A_1 - N_{f1} \\ N_{f1} \end{pmatrix}^2 \begin{pmatrix} \frac{1}{\alpha}A_1 - 2N_{f1} \\ N_{b1} \end{pmatrix}^2 \begin{pmatrix} \frac{1}{\alpha}A_1 - 2N_{f1} - N_{b1} \\ N_{g1} \end{pmatrix}^2 \begin{pmatrix} \frac{1}{\alpha}A_1 - 2N_{f1} - N_{b1} - N_{g1} \\ N_{g1} \end{pmatrix}^2. \tag{30}$$

B. Case of an infinite number of cells

Now we consider another special case consisting of an infinite number of cells or vesicles periodically lying along a straight line on the flat substrate. Due to the features of periodicity and symmetry, only one cell in simultaneous contact with two neighboring cells and the matrix needs to be considered. The expressions of U, Γ, W and S become

$$U = -N_{b0}E_a - N_{b1}E_a, \tag{31}$$

$$\Gamma = \frac{1}{2}N_{b0}\xi_{b0}(h_0 - L_{b0})^2 + \frac{1}{2}N_{b1}\xi_{b1}(h_1 - L_{b1})^2 + \frac{1}{2}N_{g0}\xi_g(h_0 - L_g)^2 + \frac{1}{2}N_{g1}\xi_g(h_1 - L_g)^2, \tag{32}$$

$$\Delta W = \gamma \Delta A, \tag{33}$$

$$\Omega = \Omega_1 \times \Omega_2, \tag{34}$$

where,

$$\Omega_1 = \begin{pmatrix} \frac{1}{\alpha}(A_t + \Delta A - A_0 - A_1) \\ N_t - N_{b0} - N_{f0} - N_{b1} - N_{f1} \end{pmatrix} \begin{pmatrix} \frac{1}{\alpha}(1-\rho_0)A_0 \\ N_{f0} \end{pmatrix} \begin{pmatrix} \frac{1}{\alpha}\rho_0 A_0 \\ N_{b0} \end{pmatrix} \begin{pmatrix} \frac{1}{\alpha}(1-\rho_0)A_0 - N_{f0} \\ N_{g0} \end{pmatrix} \times \begin{pmatrix} \frac{1}{\alpha}(A_t + \Delta A - A_0 - A_1) - (N_t - N_{b0} - N_{f0} - N_{b1} - N_{f1}) \\ N_g - N_{g0} - N_{g1} \end{pmatrix}, \tag{35}$$

$$\Omega_2 = \begin{pmatrix} \frac{1}{\alpha}A_1 \\ N_{f1} \end{pmatrix} \begin{pmatrix} \frac{1}{\alpha}A_1 - N_{f1} \\ N_{f1} \end{pmatrix} \begin{pmatrix} \frac{1}{\alpha}A_1 - 2N_{f1} \\ N_{b1} \end{pmatrix} \begin{pmatrix} \frac{1}{\alpha}A_1 - 2N_{f1} - N_{b1} \\ N_{g1} \end{pmatrix} \begin{pmatrix} \frac{1}{\alpha}A_1 - 2N_{f1} - N_{b1} - N_{g1} \\ N_{g1} \end{pmatrix}. \tag{36}$$

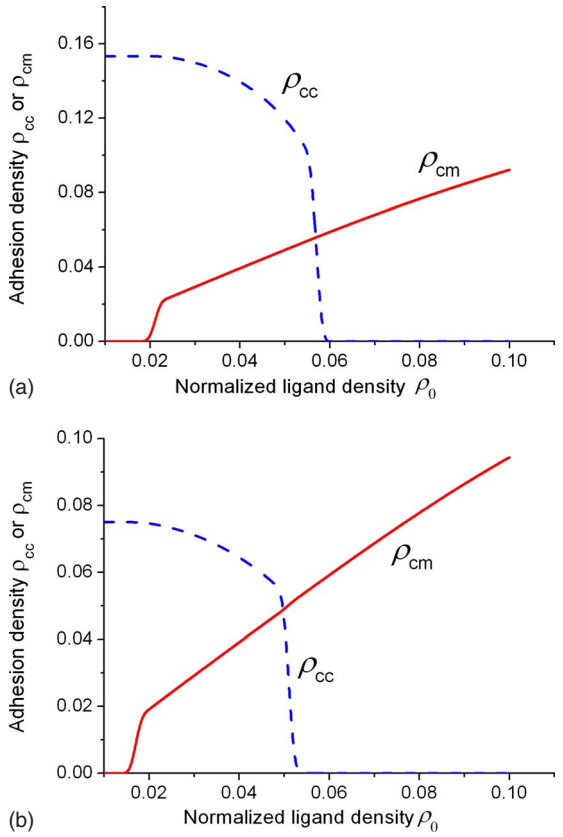

FIG. 7. (Color online) Variations of the cell-matrix adhesion density ρ_{cm} and cell-cell adhesion density ρ_{cc} with respect to the ligand density ρ_0 on the matrix. (a) The case of three cells, and (b) the case of an infinite number of cells.

C. Results and discussions

By minimizing the Gibbs free energy function of the system containing N_{cell} cells, one can examine the effects of various factors on the competitive cell-cell and cell-matrix adhesions, as described in Sec. IV. Additionally, the dependence relationships of the adhesion densities ρ_{cc} and ρ_{cm} on the ligand density ρ_0 on the matrix are plotted in Figs. 7(a) and 7(b) for $N_{cell}=3$ and $N_{cell}=\infty$, respectively. In both the cases, there exist a critical ligand density below which no cell-matrix adhesion happens and another above which no cell-cell adhesion takes place. In other words, the simultaneous occurrence of cell-cell and cell-matrix adhesions happens only in the medium range of ligand density on the matrix. Comparing Figs. 2(c), 7(a), and 7(b) leads to the conclusion that the cell-cell adhesion density ρ_{cc} decreases with increasing cell number N_{cell}, when there exists no cell-matrix adhesion. It is evident that with the increase of N_{cell}, the average adhesion area of each cell will increase. Correspondingly, the cell-cell adhesion density will decrease because the number of receptors on each cell is constant.

VI. CONCLUSIONS

Based on the biophysical mechanisms of cell adhesion, we have developed a theoretical model to study the competition between cell-cell and cell-matrix adhesions. It allows us to account for some key factors that influence the competitive process, e.g., the binding energy of receptors and ligands, the properties (equilibrium lengths and spring constants) of glycocalyx molecules and bonds, the elasticity of cell membranes, the mixing entropy associated with the mobility and the finite number of receptors and repelling molecules, and the density of immobile ligands on the matrix.

The Monte Carlo method is utilized to account for the stochastic nature of the adhesion process and to study the competition between cell-cell and cell-matrix adhesions. The results show that the density of the immobilized ligands on the matrix plays an important role in the competitive adhesions. There are a minimal ligand density for the existence of cell-matrix adhesion and another critical ligand density above which cell-cell adhesion will not take place. The cell-cell adhesion density is generally much larger than the cell-matrix adhesion density when both cell-cell and cell-matrix adhesions exist. It was also found that glycocalyx interaction has a greater influence on cell-matrix adhesion than on cell-cell adhesion and that the cell-cell adhesion density decreases with the increase in the cell number.

Finally, it should be pointed out that some other factors (e.g., cytoskeleton, thermal fluctuations, and substrate morphology [46]) that also influence cell adhesion have not been taken into account in the present model. In addition, since the adhesion processes of multiple cells are often in dynamic and nonequilibrium state, some modifications are necessary further to capture some more realistic physical mechanisms. In spite of the limitations, the present study is helpful for understanding some important experimental phenomena related to multiple adhesive cells on a matrix.

ACKNOWLEDGMENTS

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