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Nonlinear mechanical modeling of cell adhesion

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Abstract

Cell adhesion, which is mediated by the receptor–ligand bonds, plays an essential role in various biological processes. Previous studies often described the force–extension relationship of receptor–ligand bond with linear assumption. However, the force–extension relationship of the bond is intrinsically nonlinear, which should have significant influence on the mechanical behavior of cell adhesion. In this work, a nonlinear mechanical model for cell adhesion is developed, and the adhesive strength was studied at various bond distributions. We find that the nonlinear mechanical behavior of the receptor–ligand bonds is crucial to the adhesive strength and stability. This nonlinear behavior allows more bonds to achieve large bond force simultaneously, and therefore the adhesive strength becomes less sensitive to the change of bond density at the outmost periphery of the adhesive area. In this way, the strength and stability of cell adhesion are soundly enhanced. The nonlinear model describes the cell detachment behavior better than the linear model.

Keywords: Cell adhesion; Focal adhesions; Nonlinear; Receptor–ligand bonds; Force–extension relationship; Bond density

1. Introduction

In the past 2 decades, considerable efforts have been made to understand the behavior of cell adhesion due to its significance in biological processes, such as cell growth, motion, differentiation, morphogenesis, and the generation of intracellular signals (Brown, 1997; Gimbrone et al., 1997; Gumbiner, 1996). It is increasingly evident that mechanotransduction between cells and their environments based on cell adhesion regulates the gene expression and cell fate (Geiger and Bershadsky, 2001). Cell adhesion is a very complex dynamic biological process. The binding of the cell to a surface occurs through specific intermolecular interactions: receptor molecules on the surface of cell membrane bind to the complementary ligand molecules on the substrate. Focal adhesions (FAs) are the sites of tightest adhesion to the underlying extracellular matrix and provide a structural link between the actin cytoskeleton and the extracellular matrix, and account for roughly 5–15% of the entire adhesive zone (Wayner et al., 1991). During the formation of FA, bound receptors associate with the actin cytoskeleton and cluster together rapidly. The clustering of adhesion receptors is an essential step in the development of FAs. Because of these clusters, such as focal complex and FAs, the bond distribution in the adhesive zone is non-uniform (Tawil et al., 1993; Wehrle-Haller and Imhof, 2002).

Various theoretical models have been developed for understanding and quantifying the biophysics of cell adhesion, the readers are referred to the review papers (Gracheva and Othmer, 2004; Sengers et al., 2007; Zhu, 2000; Zhu et al., 2000). Evans (1985a, b) examined the mechanics of cell adhesion with one-dimensional (1D) peeling model by assuming the bonds between membranes were continuously or discretely distributed throughout the adhesive zone. For a continuum distribution, he found that the tension required to detach the membrane is equal to the tension induced by adhesion. However, this is not the case for discrete attachments, where the tension for detachment is significantly larger than the tension generated during attachment. Ward and Hammer (1993) examined how receptors clustering, driven by cytoskeletal cross-linking,

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affect cell-substrate attachment strength, with 'peeling' and 'fracture' models. Their work predicted large increases in adhesive strength resulting from receptor clustering and formation of FAs. Employing the methodology developed by Dembo et al. (1988), Ward et al. (1994) calculated the rate of detachment of a membrane containing a patch of high receptor density and found that peeling was inhibited by increasing the bond density of receptor cluster, whereas the chemical or mechanical properties of receptor–ligand bonds within the patch can further influence the stability of cell adhesion. The complex molecular structure formed at FA increases the bending stiffness of cell, and Martinez et al. (2004) showed that cortex stiffening significantly influences the force required for detachment. Spatz and colleagues (Arnold et al., 2004; Cavalcanti-Adam et al., 2005; Walter et al., 2006) used novel micro- and nano-structured materials to study the FA of living cells. They found that the bond distribution induced by nanopatterned surface plays a central role in the formation of FA of the cell.

Besides the peeling model, many other models have been proposed to describe cell adhesion and cell mobility. DiMilla et al. (1991) adopted a highly simplified cell model in which cytoskeleton is modeled by a series of viscoelastic elements that transmit the force generated by cytoskeletal elements to the adhesion bonds modeled as springs. Palsson and Othmer (2000) developed a model for the movement of *Dictyostelium discoideum* cells, either as individuals or collectively as aggregates. There a cell is modeled as a deformable ellipsoid of constant volume that contains a nonlinear spring in parallel with a Maxwell element along each axis of the ellipsoid. In Gracheva and Othmer (2004)'s work the cell was modeled as a linear viscoelastic material, with an active protrusive stress due to actin polymerization at the leading edge and cytoskeleton elasticity, active stress due to myosin and drag induced by adhesion via integrin, all dependant on the position along the cell.

In previous studies, receptor–ligand bonds were often modeled as linear elastic springs, with certain elastic coefficient. However, the mechanical behavior of the bonds is generically nonlinear. In addition, the deformation of bonds along stretching pathways is correlated with the conformational changes of receptor and ligand (Evans and Ritchie, 1997; Marshall et al., 2003, 2005). Therefore, the linear assumption is too simple to take into account the complex deformation of the bonds as well as the cell membrane and cytoskeleton. Experimental and numerical studies showed that the force–extension relationship of bond is highly nonlinear, especially at the threshold of the bond breaking, e.g. that of P-Selectin and ligand PSGL-1 bond (Hanley et al., 2003), and that of adhesion protein CD2-CD58 complex in T-lymphocyte (Bayas et al., 2003). Therefore, an important question arises that how the nonlinearity of the force–extension relationship of bonds affects the adhesive strength of cell. Recent studies showed that the nonlinear behavior of atomic/molecular bond can significantly influence the fracture strength of materials and adhesive strength between two contact surface (Buehler et al., 2006; Gao and Ji, 2003; Gao and Yao, 2004; Gao et al., 2003; Ji and Gao, 2004a, b; Yao and Gao, 2006). However, there are few studies considering the nonlinear property of the receptor–ligand bond which is crucial for understanding the mechanical behavior of cell adhesion. In present study, a nonlinear 1D peeling model was developed, and the influence of nonlinear mechanical behavior of bonds on the adhesive strength was then studied at three different types of bond distribution. We find that the nonlinear force–extension relationship of bonds can significantly increase the adhesive strength, and in particular, it makes the strength of cell adhesion be less sensitive to the change of bond density at the outmost periphery of the adhesion zone.

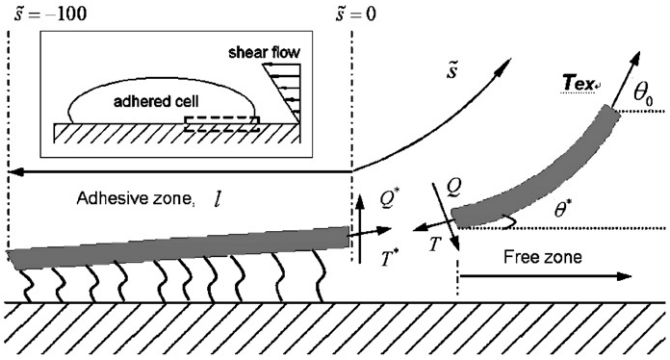

Fig. 1. One-dimensional peeling model for cell adhesion. The intensive forces on the cross section of membrane include an internal tension T that acts tangent to the plane of the membrane surface and a transverse shear Q that acts normal to the membrane, plus the adhesive forces. The inlet shows an adhered cell on the substrate in shear flow.

2. A nonlinear peeling model

Cell membrane is treated as an elastic shell that can only have bending deformation.[1] A force T_{ex} acted away from the adhesive zone (see Fig. 1) is the force resultant of external forces acted on the part of the membrane considered in the model. These external forces can come from different sources for different cells. For example, for the endothelial cell in blood vessel T_{ex} is generated by the shear flow of blood. Although the geometry of cell is irregular and has three spatial dimensions, for computational simplicity here we consider an axisymmetric shell. Thus the problem can be studied in the meridional plane normal to the edge of the adhesive zone that depends only on the curvilinear coordinates (s, θ) of the membrane, as a 1D problem. The adhesive stresses acting along the receptor–ligand bonds are assumed to be normal to the membrane surface. The membrane is divided into two

[1]Previous works (Evans, 1985a, b; Freund and Lin, 2004; Gao et al., 2005; Ward et al., 1994) showed that the bending energy is dominant in the elastic energy of cell membrane, where the stretching energy/deformation can be negligible.

zones, i.e. the free zone where the membrane is not subject to attractive stresses, and the adhesive zone where the membrane is held together with substrate by attractive stresses. The general approach for solving this problem is to analyze the membrane mechanics for each zone separately and then to require continuity of the solutions at the interface between the two zones. In the linear modeling (Dembo et al., 1988; Evans, 1985a; Martinez et al., 2004; Ward et al., 1994), cell detachment occurs when the bond force at the leading edge of the cell exceeds the maximum bond force, then the mechanical instability ensues and the cell detaches from the substrate. However, unlike the linear model above, the nonlinear model allows the bonds to continue resisting the detachment after its bond force exceeds the maximum bond force, and a new criterion is required for the detachment of cell.

In Fig. 1, l is the dimensionless length of the entire adhesive zone normalized by the bond extension L_m ($l = S/L_m$), where S is the length of the entire adhesive zone, and $L_m = 20\,\text{nm}$ which is shown in Table 1. Therefore, the unit of the length is 20 nm, and the entire length S of the adhesive zone is equal to 2 μm when $l = 100$.

The local mechanical equilibrium of the membrane in the adhesive zone is given by the following equations:

$$\frac{dT}{ds} - QK = 0, \quad TK + \frac{dQ}{ds} = -fn, \tag{1}$$

where T is the internal tension in membrane, Q is the internal transverse shear force, K is the local curvature, f is the adhesive bond force of a single bond, and n is the bond density. If the bond force $f = 0$, Eq. (1) degenerate to the equilibrium equations for the membrane of free zone, and can be further simplified into (Martinez et al., 2004),

$$T = -\frac{d^2T}{d\theta^2}. \tag{2}$$

The solutions for the free zone are functions of the local angle θ, the external force T_{ex}, the macroscopic angle θ_0 and the bending modulus B of the membrane, shown as follows (Evans, 1985a)

$$\begin{cases} T_f = T_{ex}\cos(\theta_0 - \theta), \\ Q_f = T_{ex}\sin(\theta_0 - \theta), \\ (K_f)^2 = \dfrac{2T_{ex}}{B}[1 - \cos(\theta_0 - \theta)]. \end{cases} \tag{3}$$

In the adhesive zone, according to the geometric condition (the bonds are normal to the membrane), the local angle θ can be related to the bond extension L, i.e. $dL/ds = \tan\theta(1 + L(d\theta/ds))$ (Martinez et al., 2004). The arc length s, bond extension L, bond density n, internal tension T and bond force f are normalized as (Martinez et al., 2004)

$$\tilde{s} = \frac{s}{L_m}, \quad \tilde{L} = \frac{L}{L_m}, \quad \tilde{n} = \frac{n}{n_0}, \quad \tilde{T} = \frac{TL_m^2}{4B}, \quad \tilde{f} = \frac{f}{f_0}, \tag{4}$$

where L_m is the bond extension for maximum bond force (see Fig. 2), n_0 is the average bond density (a constant) in the adhesive zone, and f_0 is a force constant for a specific kind of receptor–ligand bond (equal to ekL_m). For the nonlinear mechanical model, the critical bond extension for bond failure L_{fail} is not equal to L_m, but much larger than L_m, which we will show later. Substituting Eq. (4) into the equilibrium equations (Eq. (1)) leads to the following nonlinear differential equations for the local angle θ and the

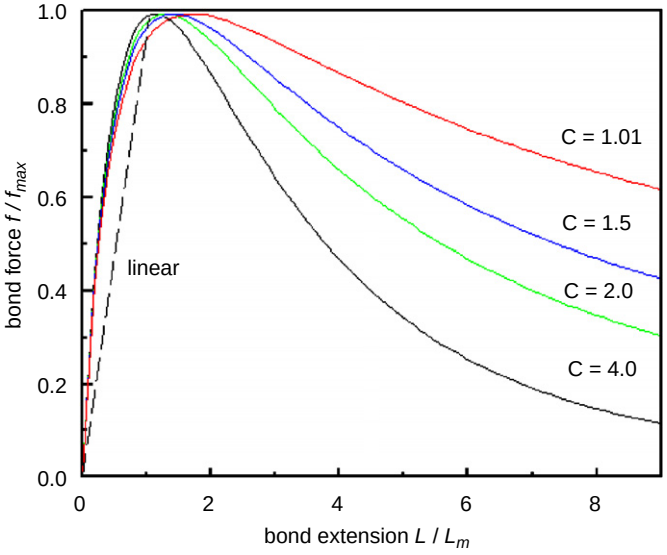

Fig. 2. The bond force–extension relationship. The dashed line is for a linear force–extension relationship, and the other four curves with long tails are described by the nonlinear force–extension relationship (Eq. (6)) with different degrees of nonlinearity, characterized by the parameter C. The bond forces of these curves have the same peak value.

Table 1
Main parameters

Parameter	Definition	Physiological range	Value used in the paper	Source
B	Bending modulus of cell membrane	$0.4–4 \times 10^{-12}$ ergs	10^{-12} ergs	Evans (1983); Ward et al. (1994)
k	Elastic constant of molecular bond	10^{-2}–10^{1} dyn/cm	10 dyn/cm	Bell et al. (1984)
n_0	Bond density	10^{6}–10^{12} cm^{-2}	10^{8} cm^{-2}	Bell et al. (1984)
L_m	Critical bond extension[a]	10–100 nm	20 nm	Bell et al., (1984); Ward et al. (1994)

[a]The bond extension for the maximum bond force.

internal tension $\tilde{T}$,

$$\begin{cases} \dfrac{d^3\theta}{d\tilde{s}^3} - 4\tilde{T}\dfrac{d\theta}{d\tilde{s}} + \tau\tilde{n}\tilde{f} = 0, \\ \dfrac{d\tilde{T}}{d\tilde{s}} + \dfrac{1}{4}\dfrac{d^2\theta}{d\tilde{s}^2}\dfrac{d\theta}{d\tilde{s}} = 0, \\ \dfrac{d\tilde{L}}{d\tilde{s}} = \tan\theta\left(1 + \tilde{L}\dfrac{d\theta}{d\tilde{s}}\right), \end{cases} \tag{5}$$

where $\tau = f_0 n_0/(4B/L_m^3)$ is a dimensionless parameter.

Although the 1D peeling model is simple, but it allows us to consider the nonlinear mechanics of bond and the inhomogeneous distribution of bond in the adhesive zone, which has not been studied by previous works. To model the nonlinear mechanics of bond deformation, we adopt the force–extension relationship of bonds as (Gao and Ji, 2003)

$$f(\tilde{L}) = f_0 C\ \ln[1 + \tilde{L}/C]/[1 + \tilde{L}/C]^C, \tag{6}$$

where C is a dimensionless parameter characterizing the nonlinearity of the force–extension relationship of bond and the ability of bond having large deformation, as shown in Fig. 2. Small C value ($1 < C < \infty$) corresponds to strong ability of having large deformation. The peak value of bond force of the nonlinear relationship is assumed to be equal to the maximum bond force of the linear relationship. Eq. (6) describes a cohesive hyperelastic behavior of bond deformation, which has been observed by recent experiments (Marshall et al., 2005) and also been demonstrated by MD simulations (Hanley et al., 2003; Lu and Long, 2004). In this paper, the bond distribution in the adhesive zone is described by the bond density function $\tilde{n}(s)$.

Seven boundary conditions are needed for the solution of Eq. (5), where five arising from the orders of the differential equations and two arising from the unknown parameters T^* and θ^* at the origin (the leading edge of the adhesive zone), shown in Fig. 1. According to the continuity of the internal tension, shear force and moment at the origin, we get five boundary conditions: the first three come from Eq. (3) and the last two come from the continuity of local angle and bond extension, shown as Eq. (7),

$$\begin{cases} \tilde{T}(0) = \tilde{T}^*, \\ \left(\dfrac{d\theta}{d\tilde{s}}|_{\tilde{s}=0}\right)^2 = 8\tilde{T}^*[1 - \cos(\theta_0 - \theta^*)] \Big/ \cos(\theta_0 - \theta^*), \\ \dfrac{d^2\theta}{d\tilde{s}^2}|_{\tilde{s}=0} = -4\tilde{T}^* \sin(\theta_0 - \theta^*) \Big/ \cos(\theta_0 - \theta^*), \\ \theta(0) = \theta^*, \\ \tilde{L}(0) = \tilde{L}_{fail}, \end{cases} \tag{7}$$

where $\tilde{L}_{fail}$ is the bond extension at the origin at the critical condition of cell detachment, and $\tilde{L}_{fail} > 1$ for the nonlinear model, i.e. $L_{fail} > L_m$. The other two boundary conditions can be defined at the rear of the contact line far into the adhesive zone, i.e., at $s = -l$, namely

$$\theta = 0, \quad \text{and} \quad \frac{d\theta}{d\tilde{s}} = 0. \tag{8}$$

The differential equations of the peeling model, Eq. (5), is then simplified into a nonlinear two-point boundary value problem with two unknown parameters $\tilde{T}^*$ and θ^*. They are characterized by three parameters τ, θ_0 and C, and can be solved numerically within the adhesive zone. The length of adhesion zone AB in non-dimensional unit is equal to 100, i.e., $l = 100$, as shown in Fig. 1. The physiological range of the main parameters and their value used in our model, as well as the references, are listed in Table 1. When we choose $B = 10^{-12}$ ergs, $L_m = 20$ nm, $n_0 = 10^8\,\text{cm}^{-2}$, $k = 10$ dyn/cm, the dimensionless constant $\tau = 0.011$.

We should point out that, for the linear peeling model, the cell detachment occurs when the bond force at the origin reaches its maximum value, corresponding to a bond extension $\tilde{L}_m(0) = 1$ (Dembo et al., 1988; Evans, 1985a, b; Martinez et al., 2004; Ward et al., 1994); however, in the nonlinear model the criterion for the detachment is different, as at the critical condition the maximum bond force normally occurs at the rear of the adhesive zone instead of at the origin. In this study, a new criterion for the detachment is introduced. We define that the detachment occurs when the detaching force achieves its maximal value. Therefore, for the nonlinear model it is much more difficult to solve Eq. (5) because the position of the bond with maximum bond force is unknown, and an initial guess for the position of the bond with maximum force is needed before calculation. The shooting method is adopted for solving the problem with the two unknown parameters $\tilde{T}^*$ and θ^*. The cell strength is indicated by the detaching force $\tilde{T}_{ex}^c$, according to Eq. (3), i.e. $\tilde{T}_{ex}^c = \tilde{T}^*/\cos(\theta_0 - \theta^*)$, which is the critical tension necessary to initiate the cell peeling. This nonlinear model allows us to give insight into how the nonlinear mechanical behavior of bond deformation affects the stability of cell.

3. Results

To study the effect of nonlinear behaviors of the bond on cell adhesion, three types of bond distribution are proposed, as shown in Fig. 3. The first one is called one-step distribution (Fig. 3A), where the receptor–ligand bonds are confined in the FA zone ($-d$, 0) with bond density $\tilde{n} = 1/H$, whereas H is the ratio of the length of FA zone to that of the entire adhesive zone, i.e. $H = d/l$, and the bond density outside of the FA zone $\tilde{n} = 0$. The second one is called two-step distribution (Fig. 3B), where the local bond density in the FA zone (10% of the entire adhesive zone), $\tilde{n} = D(1 \leqslant D \leqslant 4)$, and the bond density outside the FA zone has the following form:

$$\tilde{n} = (1 - D \times H)/(1 - H). \tag{9}$$

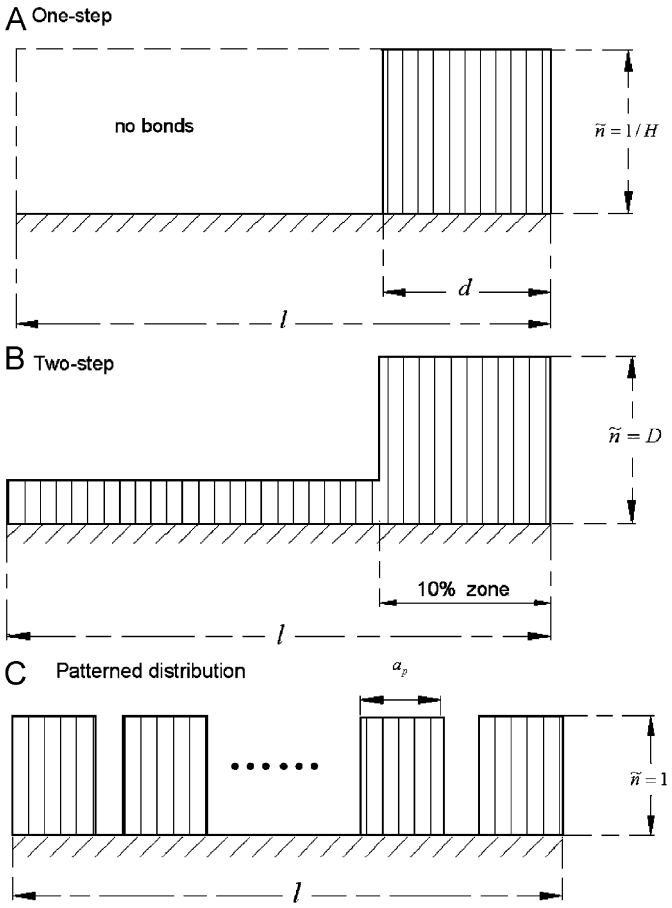

Fig. 3. Three types of bond distribution. (A) One-step bond distribution; (B) two-step bond distribution; (C) patterned bond distribution.

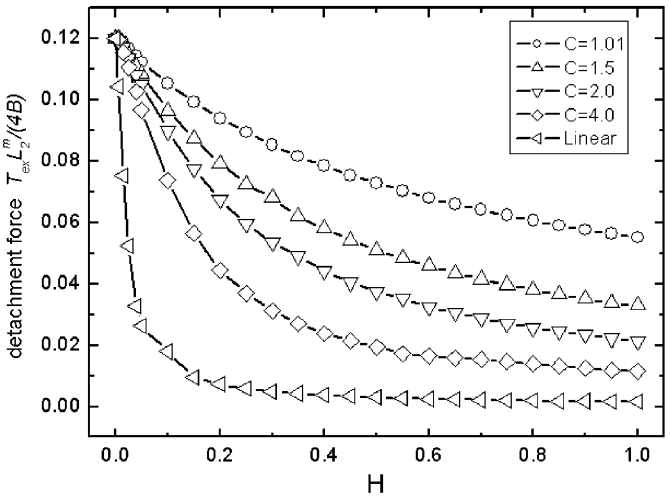

Fig. 4. The relationship between detaching force $\tilde{T}_{ex}^{c}$ and H for linear and nonlinear bond relationship (as a function of C for the nonlinear relationship). In the calculation, we choose $\tau = 0.011$, $\theta_0 = \pi/4$.

It should be noted that the average bond density in the entire adhesive zone is kept constant ($\bar{n} = 1$) for the above two kinds of bond distributions.

Fig. 3C shows the third one, called the patterned bond distribution, where M denotes the number of the islands, and $M > 2$. The bond density of each island is $\tilde{n} = 1$. The length of each island is a_p, and $\varphi = a_p M/l$, is the ratio of the summation of the length of islands to the entire length of adhesive zone. In this study, φ is kept a constant value of 2/3, and we change the island number M to study its effect on the adhesive strength.

3.1. One-step bond distribution

We firstly study the effect of the nonlinearity of the force–extension relationship on the adhesive strength for different sizes of FA zone (H) with the one-step bond distribution. The adhesive strength for the linear model as well as the nonlinear model with four different C values is calculated. The dependence of the detaching force on parameter C as a function of H is illustrated in Fig. 4. It is shown that the nonlinearity influences the adhesive strength significantly. It is also noted that the detaching force $\tilde{T}_{ex}^{c}$ decreases with the increase of H value for both linear and nonlinear models due to the decreases of bond density at the periphery (the total bond number is kept constant), displaying a clear periphery dependence. The reason is that the detaching force largely depends on the bond density at the periphery region of the adhesive zone because the bonds at the periphery deform significantly and sustain most of the external load. We find that the decreasing rate of the detaching force of the nonlinear model is much slower because of the nonlinear behaviors of bond. The underlying mechanism is that the nonlinear bond behavior can significantly reduce the stress concentration at the periphery region so that the detaching force becomes less sensitive to the variation of bond density due to the change of H value.

For the linear bond force–extension relationship, there's a steep decrease in the detaching force $\tilde{T}_{ex}^{c}$ at small H value, followed by a very low plateau (see Fig. 4). However, for the nonlinear relationship, the decrease of $\tilde{T}_{ex}^{c}$ becomes much slower in comparison with that of the linear relationship due to the nonlinear deformation mechanism; in addition, the detaching force increases with the decrease of C value. The underneath mechanism includes two aspects: (a) for the nonlinear bond, not only the bonds at the outmost periphery resist the detachment, but also the bonds far from the periphery region contribute to the adhesive strength; (b) the nonlinear force–extension relationship can also increase the adhesion energy effectively, in particular at small C value. In contrast, the adhesive strength of the linear model is very sensitive to the bond density at the periphery, showing a stronger periphery dependency, as shown in Fig. 4.

We note that the convergence of the five curves as $H \to 0$ is of particular interest, i.e. the detaching forces of the five curves have the same value of $\tilde{T}_{ex}^{c} = 0.12$. This convergence arises from the fact that all the bonds concentrate at the outmost periphery position, and they reach their maximum

bond force simultaneously and behave as a single bond. Consequently, the detachment of adhesive zone is analogy to the breakage of a single bond, and the detaching force is equal to the peak value of the force–extension relationship multiplied by the total bond number. The limit of $H \to 0$ represents the ideal maximum adhesive strength, where all the bonds break simultaneously and the adhesion achieves the highest stability and robustness. However, it is not practical to realize that all the bonds cluster together at the outmost periphery. There are a very small percentage of bonds distributing at the outmost periphery. The linear behavior only allows this small percentage of bonds to achieve maximum bond force. In contrast, the nonlinear mechanical property of bond allows more bonds in a larger area (than the outmost periphery) to approach their maximum bond force simultaneously and makes the cell adhesion robust and stable.

The robustness of nonlinear force–extension relationship can be understood from the profiles of the bond extension and force, e.g., at $C = 1.5$, with $H = 10\%$ and 50%, respectively, as shown in Fig. 5. In each panel, the upper illustration is the bond extension profile, and the below one is the bond force profile. We can see that the nonlinear force–extension relationship allows the bonds to provide adhesion force even when their deformations are larger than the bond extension L_m which corresponds to the maximum bond force, where the length of L_m is indicated by the dot line in Fig. 5; in contrast, the bond force will become zero when $L > L_m$ for the linear bond. We also note that the maximum bond force does not occur at the origin as the linear model does, instead it occurs somewhere away behind the origin. Therefore, the nonlinear behavior allows more bonds to involve in the resistance to the detachment of cell adhesion, which is analogy to the roles of the plastic deformation in crystals which makes the fracture strength of crystals be insensitive to the flaw as more atoms around the crack tip can take part in the resistance to the growth of crack.

To compare with the recent experiments (Gallant et al., 2005), we calculate the adhesive force for an adhesive zone of 1 μm in length containing a maximum of 3000 bonds (Gallant and Garcıa, 2007; Gallant et al., 2005). As expected, the nonlinear model agrees better with the experimental data in comparison with the linear peeling model, shown in Figs. 6A and B. In the linear model, the adhesive strength only depends on the bonds at the outmost periphery because these bonds take critical role in resisting the detachment. However, both the present nonlinear model and the model of Gallant and Garcıa (2007) show that the bonds outside of periphery also take important roles in the adhesive strength due to the generic nonlinear behavior of adhesive bonds. Fig. 6C shows the distribution of bond force predicted by our model, where the bond force outside of periphery still keep high value in comparison with the linear model, which is consistent with the results of Gallant and coworkers (Gallant and Garcıa, 2007; Gallant et al., 2005).

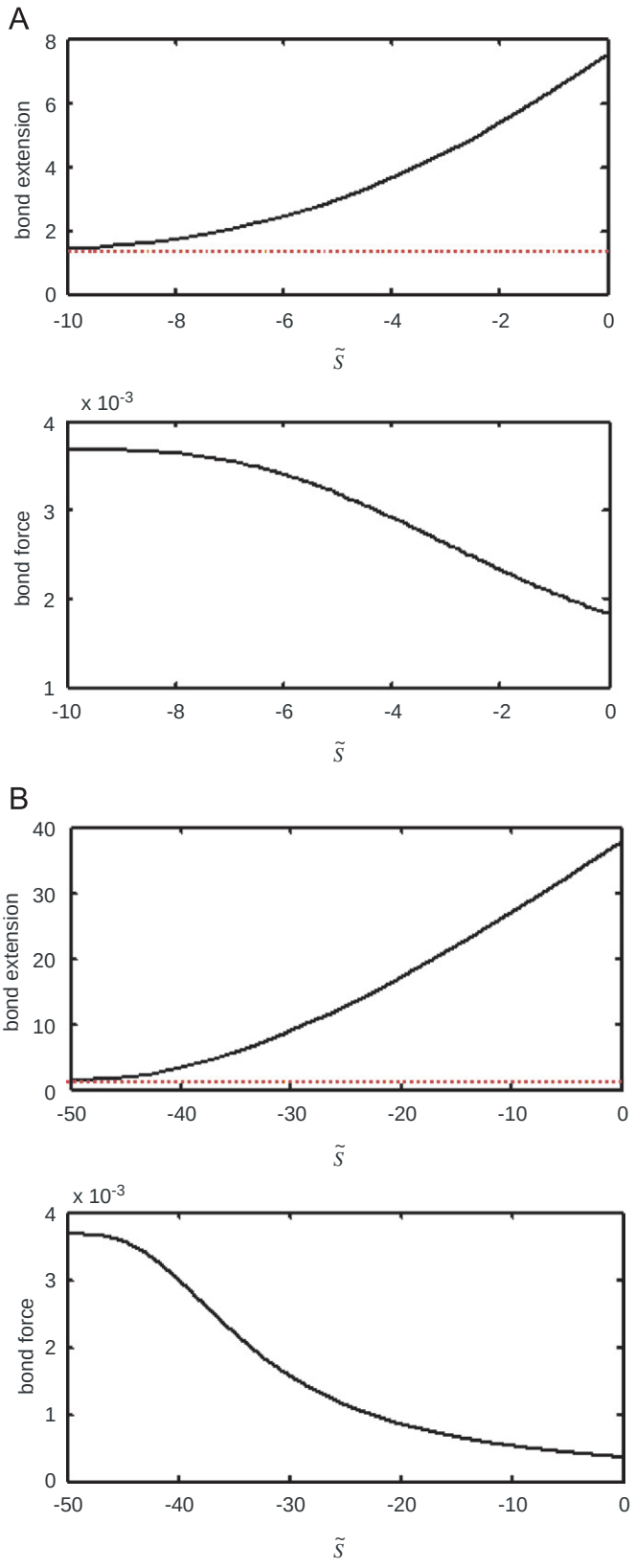

Fig. 5. Bond extension and force profiles for different size of adhesive zone at $C = 1.5$. (A) $H = 10\%$; (B) $H = 50\%$. The dot line in each panel indicates the value of bond extension corresponding to the maximum bond force. The bond force is also normalized by $4B/L_m^2$ in all the bond force profiles (Figs. 5, 8 and 10).

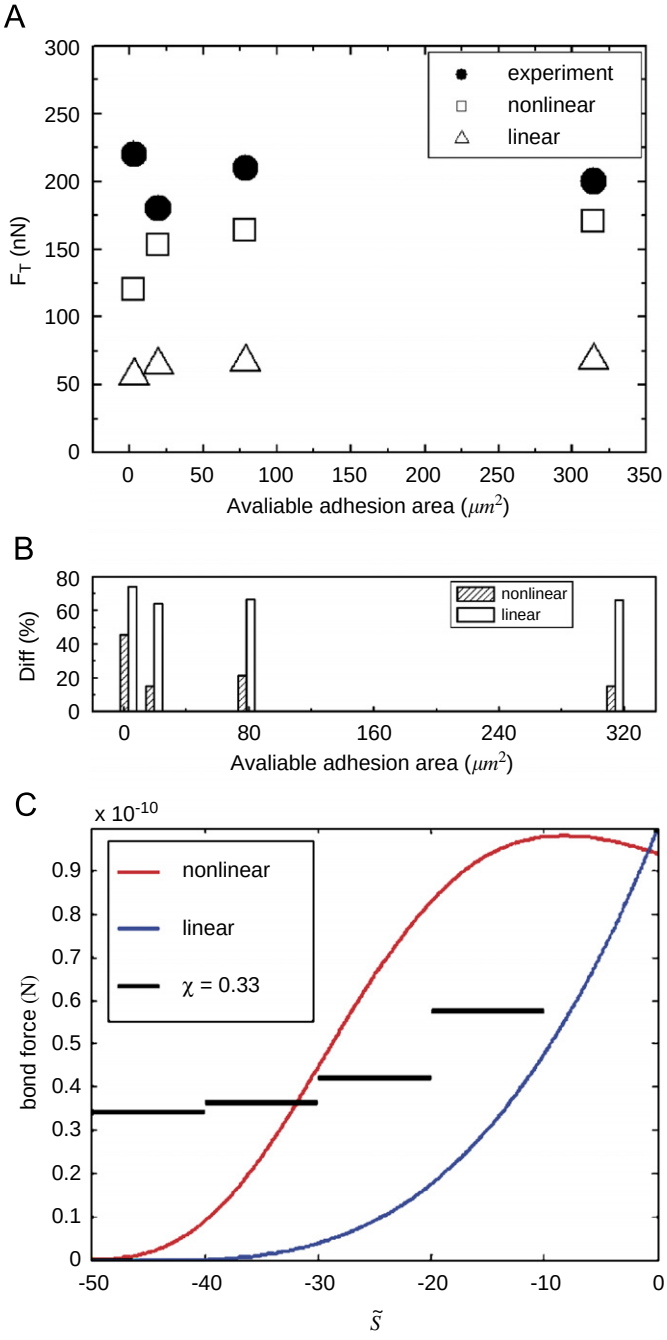

Fig. 6. The comparison of the adhesive force predicted by the nonlinear model with the experimental data (Gallant et al., 2005). (A) Adhesive force F_T computed from experimental measurements of adhesion strength (Gallant et al., 2005), the linear model and nonlinear model. (B) The difference of the predicted results of the linear and nonlinear models from the experiment results. (C) The bond force distribution throughout the adhesive zone, for Gallant and Garcia's model at $\chi = 0$, 0.33 (Gallant and Garcıa, 2007), the linear model and nonlinear model. The results show that the nonlinear model predicts a more consistent bond force distribution with the model of Gallant and Garcia in comparison with the linear model.

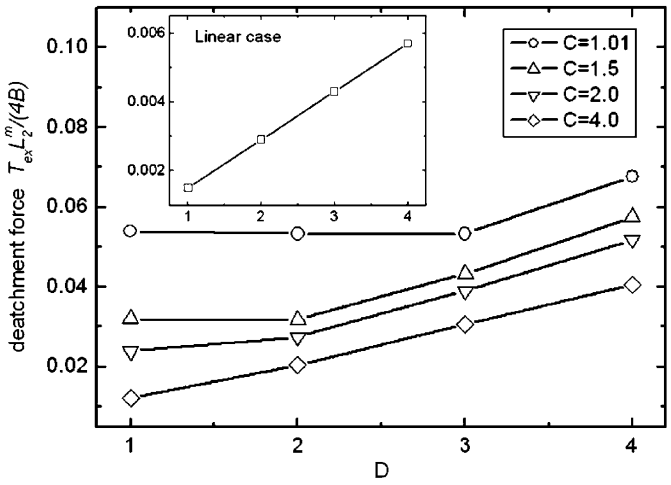

Fig. 7. The dependence of the detaching force $\tilde{T}_{ex}^c$ on parameter C as a function of D. From the top to the bottom, $C = 1.01$, 1.5, 2, 4, respectively. The inset shows the results of the linear relationship. In the calculation, we choose $\tau = 0.011$, $\theta_0 = \pi/4$.

3.2. Two-step bond distribution

To further examine the effect of the nonlinear relationship on the strength of cell adhesion, we designed a two-step bond distribution to model the effect of bond distribution associated with the FA. Unlike the one-step bond distribution, the bond density outside the FA is not equal to zero (see Eq. (9)). The detaching force is calculated based on this bond distribution. It can be seen from Fig. 7 that the relationship between the detaching force $\tilde{T}_{ex}^c$ and bond density D of the FA zone is approximately a linear relation for both the linear bond and the nonlinear bond with low degree of nonlinearity (higher C value, e.g. $C > 2$). In addition, the detaching force increases as the local bond density D increases, and high degree of nonlinearity (lower C value, e.g., $C = 1.01$) corresponds to high detaching force. But for high degree of nonlinearity, the detaching force increases slower and is less sensitive to the local bond density D, and even keeps approximately a constant value in the range of $1 \leqslant D \leqslant 3$ (see Fig. 7; in reality, the difference in bond density between the FA and the non-FA zone can not be so high due to energy unfavorable for high difference in chemical potential). This again shows the robustness of cell adhesion due to the nonlinear behavior of bond deformation. The result indicates that nonlinear behavior allows the bonds to resist the detachment effectively even when they are a bit far from the leading edge (origin). Therefore, the detaching force is less sensitive to the variation of bond density in the periphery region in comparison with the stronger periphery dependency of the linear model.

The insensitivity can be understood further from the bond extension and force profiles in Fig. 8. For example, in the case of $C = 1.5$, the maximum bond force for the maximum detaching force occurs not at the origin, but at location $s = -90$ and -15 for $D = 1$ and 3, respectively ($D = 1$ means the receptor–ligand bonds are uniformly distributed throughout the adhesive zone). Bonds in

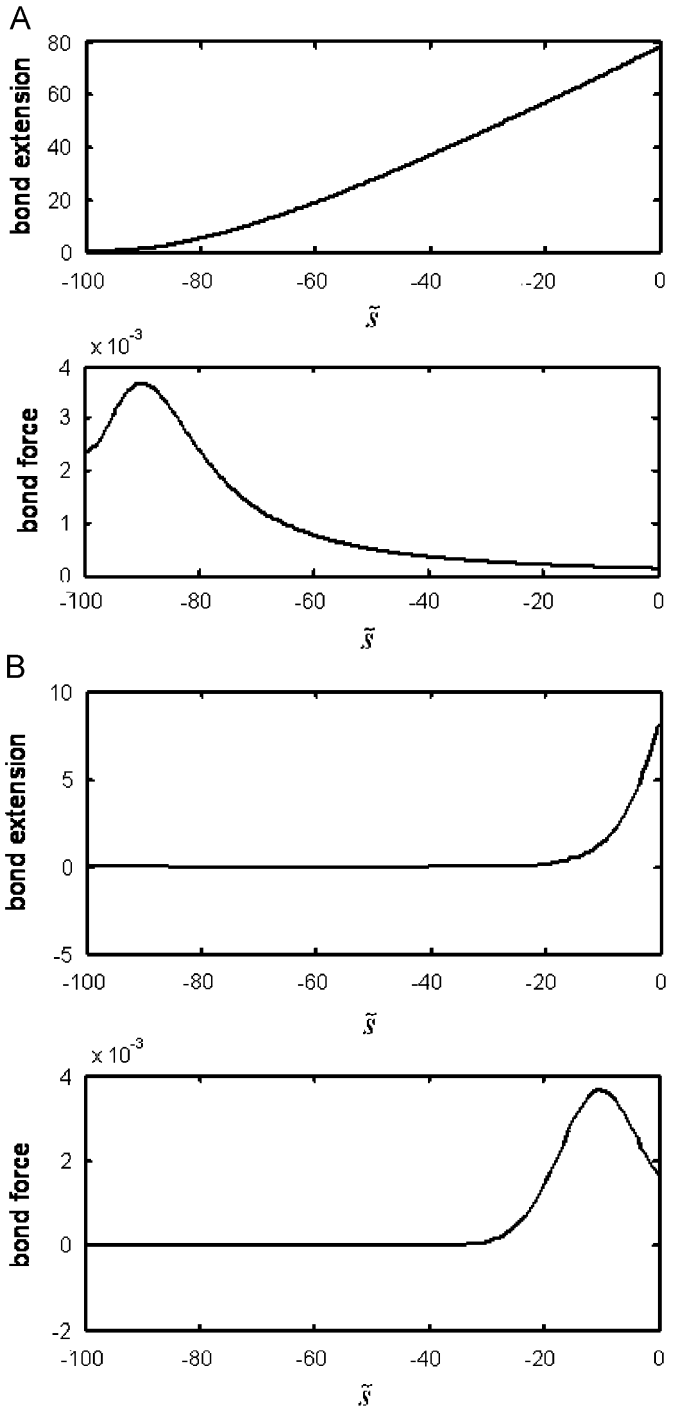

Fig. 8. Bond extension and force profiles for different values of local bond denstiy D at $C = 1.5$. (A) $D = 1$; (B) $D = 3$.

a larger area around the periphery can contribute to the resistance to the detachment in comparison with the linear model, e.g. at $D = 1$, almost all the bonds in the adhesive zone are elongated at the detachment of the cell, as shown in Figs. 8A and B. However, the linear relationship cannot achieve this because the bonds can not undergo this hyperelastic deformation.

3.3. Patterned bond distribution

Currently there are several experimental studies on how substrate pattern influences the cell adhesion, e.g. Spatz and coworkers (Arnold et al., 2004; Cavalcanti-Adam et al., 2005; Walter et al., 2006) studied the effect of nano-patterned surfaces on the formation of FA and the detaching force of cell during the initial adhesion process. These experiments with patterned morphologies of matrix can help us understand how the patterned bond distribution influences the cell adhesion. For simplicity, here we use 1D periodic pattern to study the effect of patterned bond distribution on the adhesive strength. The islands in the pattern on the substrate have the same size and bond density, shown in Fig. 3C. When the number of islands M increases, the size of the islands will become smaller, but bonds density is kept constant $\tilde{n} = 1$. The bond distribution in the adhesive zone will become more uniform globally as M increases.

We have studied the effect of the patterned bond distribution on the detaching force for both linear and nonlinear relationships. The dependence of the detaching force on island number M of the nonlinear relationship ($C = 1.5$ and 2) shown in Fig. 9, is compared with that of the linear relationship in the inset. The detaching force of the nonlinear relationship firstly decreases rapidly with the increase of M at the beginning due to the decrease of average bond density in the periphery. Then, the decrease becomes slower and the value of the detaching force stays at a plateau at last, which means the detaching force becomes insensitive to the variation of M when the number of islands is larger than a threshold. We find that this transition depends on the C value: it occurs at $M = 6$ for $C = 1.5$, but at $M = 10$ for $C = 2$. Of particular interest is that the linear relationship predicts a very different dependence of detaching force on M, as shown in the inset of Fig. 9. The detaching force keeps at a constant value at the beginning of M increasing, because the area of the

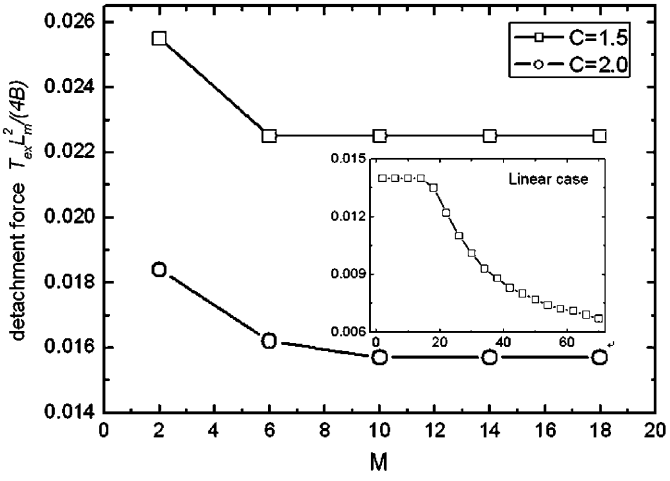

Fig. 9. The dependence of the detaching force $\tilde{T}_{ex}^{c}$ on the island number M as a function of C. In the calculation, we choose $\tau = 0.011$, $\theta_0 = \pi/4$.

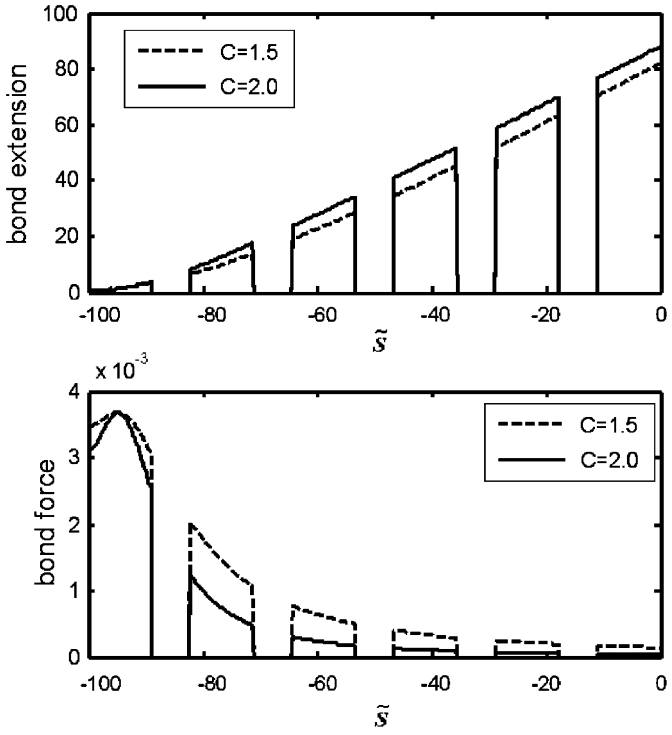

Fig. 10. Bond extension and force profiles for different values of C at $M = 6$.

outmost island is larger than the size of the periphery region for the linear model, so the change of M will not influence the bond number in the periphery at small M. However, when the value of M exceeds a threshold value at which the size of the outmost island becomes smaller than the size of the periphery region, the detaching force starts to decrease. In addition, the detaching force cannot reach a convergent value even at very large M, e.g. $M = 40$. The physical mechanism comes from serious stress concentration at the origin for the linear model that induces stronger dependence of adhesive strength on the bonds density at the periphery.

We show that the nonlinear relationship plays a central role in the stability of cell adhesion, which makes the adhered cell more stable on the patterned substrate in comparison with the linear relationship. In Fig. 10, we plot the extension and force profiles for $C = 1.5$ and 2 at $M = 6$, respectively. We can see that when the force–extension relationship is nonlinear, the maximum value of bond force occurs at the rear region, with more bonds resisting to the detachment. Lower C value makes the bonds have a stronger resistance with larger bond force but smaller bond deformation in comparison with that of the higher C value.

4. Discussion and summary

In this work, we have studied the adhesive strength of cells with the nonlinear peeling model based on three types of bond distribution. A nonlinear force–extension relationship has been introduced into the model to describe the intrinsically nonlinear mechanical behavior of the receptor–ligand bond. Our results show that the nonlinear behavior of bonds makes the adhesion of cell more stable and robust. The nonlinear model not only predicts clearly the periphery dependence of cell adhesion as the linear model does, but also shows that this dependence is less sensitive to the change of bond density at the periphery region due to the nonlinear behavior of bonds. The higher the degree of the nonlinearity is, the less the sensitivity to the bond distribution at the periphery. In contrast, the linear model shows that the strength of cell adhesion is very sensitive to the change of bond density at the periphery region, and the detaching force decreases more rapidly with the decrease of bond density at this region. The underneath mechanism is that both the bonds in periphery region and those outside of the area undergo deformation and resist the detachment of cell because of the nonlinear behavior of the bonds. It means that the nonlinear relationship allows the coordination of the bond deformation and the redistribution of bond force among the bonds in the adhesive zone, which can drastically enhance the stability of the cell adhesion. In addition, the nonlinear behavior of the bonds not only makes the cell insensitive to the variation of bond density at the periphery region, but also enhances the adhesion energy, favored by adhesion stability.

This paper is focused on the influence of nonlinear mechanical property of receptor–ligand bonds on the adhesive strength and stability of cell by adopting a simple peeling model. The cell membrane is assumed to be a thin elastic shell in mechanical equilibrium, and the diffusion of adhesion proteins along cell membrane is not considered. This analysis is only valid at equilibrium because the non-conservative dissipation is not included in the model. Also, the present model as a 1D model, has not considered the two-dimensional (2D) distribution of adhesion molecules and the three-dimensional (3D) characters of cell deformation. In addition, our model does not take into account the deformation of cytoskeleton. However, we still adopt the viewpoint that a simple model can provide a good understanding on the problem as soon as it grasps the main features of the problem and its shortages have been fully understood. The consistence of the predication of our model with the experiments (Gallant et al., 2005) provides the validation for our model. A more complex adhesion model of considering more effects, such as diffusion of adhesive molecules and the deformation of cytoskeleton, etc. will be developed in our future study.

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