Optimum size of a molecular bond cluster in adhesion

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The strength of a bonded interface is considered for the case in which bonding is the result of clusters of discrete bonds distributed along the interface. Assumptions appropriate for the case of adhesion of biological cells to an extracellular matrix are introduced as a basis for the discussion. It is observed that those individual bonds nearest to the edges of a cluster are necessarily subjected to disproportionately large forces in transmitting loads across the interface, in analogy with well-known behavior in elastic crack mechanics. Adopting Bell's model for the kinetics of bond response under force, a stochastic model leading to a dependence of interface strength on cluster size is developed and analyzed. On the basis of this model, it is demonstrated that there is an optimum cluster size for maximum strength. This size arises from the competition between the nonuniform force distribution among bonds, which tends to promote smaller clusters, and stochastic response allowing bond reformation, which tends to promote larger clusters. The model results have been confirmed by means of direct Monte Carlo simulations. This analysis may be relevant to the observation that mature focal adhesion zones in cell bonding are found to have a relatively uniform size.

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I. INTRODUCTION

Adhesion is the principal means by which a biological cell is anchored to extracellular matrix. As such, it is an essential prerequisite for certain biological functions—for example, cell differentiation [1] or cell motility [2–5]. The noncovalent bonds formed between ligand and receptor molecules on opposing surfaces are responsible for the formation of adhesion regions which, when fully developed, are called focal adhesion zones. A striking feature of focal adhesion regions is that the maximum size they achieve, around 1 μm in diameter, is almost always the same despite their locations [6,7]. In a living cell, these adhesion clusters are subjected to disruptive forces due to, for example, shear flow in the fluid surrounding the cell or the thermally induced relative motion of the opposing surfaces. In order to function properly, the adhesion clusters must possess a certain level of strength.

Motivated by these observations, we examine an idealized model problem in which two soft elastic materials (representing a cell and the substrate, for example) are in adhesive contact over a shared interface via clusters of discrete bonds. The interface is required to transmit a certain level of force per unit area on a scale that is large compared to cluster size. Clusters are periodically distributed along the interface so as to render the determination of load transmitted per cluster specific. We address the question as to whether or not the interface strength is dependent on the cluster size under these circumstances and, if so, how to describe that dependence. We only consider focal adhesions here where cells adhere to the extracellular matrix through integrins that link intracellularly to actin filaments. According to Alberts *et al.* [8], there are three other main types of cell adhesions: namely, the adherents junctions, desmosomes, and hemidesmosomes. In principle, the analyses to be presented can be applied to any of those situations with the parameters properly chosen to represent the nature of specific type of adhesion being considered.

The study of adhesive contact between elastic spheres, as an extension of the classical contact theory of Hertz, has a rich history extending back nearly four decades. Generally speaking, if the spheres are very stiff, then the Derjaguin-Muller-Toporov (DMT) model [9] is an appropriate choice for formulating the problem. However, if the material is soft (which is the case for most biological systems), then the so-called Johnson-Kendall-Roberts (JKR) model [10] becomes applicable. With a view toward a common basis for these limiting cases, Maugis [11] developed a general mathematical formulation of the problem, for which the behaviors of the DMT and JKR models are preserved as special cases under extreme conditions.

In the models mentioned, adhesion is represented as a reduction of system free energy per unit area of interface, say, γ, as two opposing surfaces are joined. For cell adhesion, however, this concept becomes somewhat obscure because it has been convincingly demonstrated ([12–14], for example) that the enforced separation of a single ligand-receptor bond is a stochastic process and, hence, the adhesion energy in this case has meaning only from a statistical average point of view.

The strength of an adhesion cluster which consists of numerous ligand-receptor bonds was first considered by Bell [15] who proposed a framework for incorporating force in a description of bond separation. The dynamic behavior of clusters subjected to pulling forces has been studied by Seifert [16] and, recently, stochastic analyses of such systems have been conducted by Erdmann and Schwarz [17,18]. In the present study, we will show that there is an optimum cluster size for which the interface strength is maximum. As will be demonstrated below, this is a result of the interplay between geometrical stress concentrations around the cluster edges and the stochastic nature of ligand-receptor bonds.

II. MODEL FORMULATION

Consider two extended elastic solids with a shared interface as being bonded together over a periodic array of adhe-

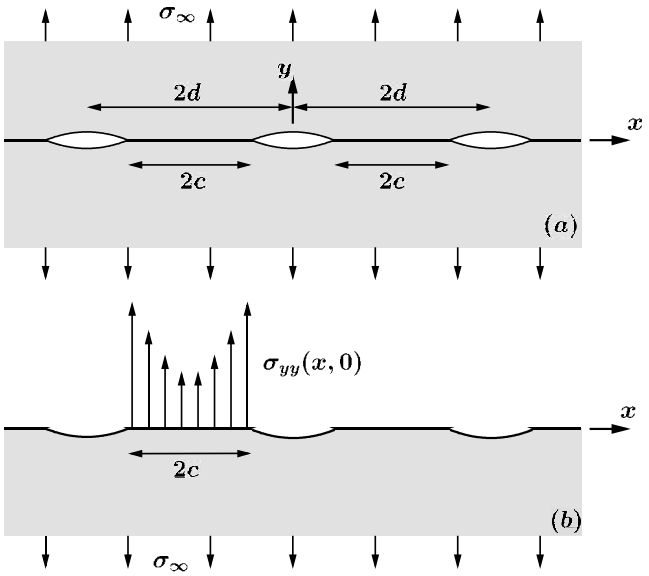

FIG. 1. Macroscopic picture of two elastic bodies in adhesive contact. The materials are connected through an array of adhesion zones along the interface, each of width $2c$, with regular spacing $2d$. Part (a) shows the joined elastic bodies being deformed by a remotely applied tensile stress σ_∞. In part (b), one of these bodies is isolated from the other so as to show schematically the nature of the distribution of stress, $\sigma_{yy}(x,0)$, transmitted across an adhesion zone.

sion zones along the interface, as depicted in Fig. 1(a). To extract the essential result to be illustrated here in the simplest way, only the two-dimensional plane strain configuration is considered. Furthermore, the two isotropic elastic materials are assumed to have the same modulus E and Poisson ratio ν. Each adhesion zone within the array has length $2c$ and the zone-to-zone period is $2d$.

A uniform stress field $\sigma_{yy}=\sigma_\infty$, acting in the direction normal to the interface and tending to separate two surfaces, is applied remotely. Under these circumstances, the force (per unit depth) transmitted by each ligament of width $2c$ is $2d\sigma_\infty$. Consequently, the average stress transmitted across each ligament is $d\sigma_\infty/c$. If the fraction $\rho=c/d$ of the interface that is bonded is fixed, then the mean stress transmitted across a ligament is also fixed. Under these conditions, we ask whether or not an optimum cluster size exists for which the interface strength is maximum. To answer this question, it is instructive to examine how load is transmitted through each adhesion patch. We proceed by first assuming that the bond itself is much stiffer than the elastic half-space, so the deformation of the bonds themselves can be neglected. After drawing some conclusions based on this situation, the possible influence of bond compliance is addressed below.

It has been assumed that the cell body can be treated as an elastic continuum and that force is transferred to an adhesion zone through this elastic continuum rather than being applied directly. A living cell with a well-developed cytoskeleton structure does indeed behave as an elastic body [19], and the external constraint imposed by adhesion can conceivably result in a distribution of stress within the cell similar to that in an elastic solid. Furthermore, there is a natural repulsion between a cell and the extracellular matrix during adhesion as a result of the glycocalyx. The repulsion in the regions between adhesion patches due to the glycocalyx is likely transferred to the adhesion zones through the cytoskeleton, as it would be in an elastic solid. While the precise mechanism of load transfer through a cell to its adhesion zones has not been measured directly, we regard diffuse load transfer, typical of elastic solids, to be among the possibilities for describing its general nature.

The configuration illustrated in Fig. 1(a) has been studied thoroughly in the context of elastic crack mechanics; see [20,21], for example. In that case, opposite surfaces are continuously bonded within each adhesion region. The constraint of adhesion and the requirement of equilibrium result in a tensile stress distribution within each adhering segment of the interface with the largest stress at the edges (in fact, square root singular at the edges in the ideal case) and with relatively small stress in the interior of the patch. Such a stress distribution is illustrated in Fig. 1(b). The strength of the singularity is commonly represented by the so-called elastic stress intensity factor, which, for the case of tension, is usually denoted by K_I.

If the period of the distribution $2d$ is several times the length of the patch $2c$, then the distribution of stress transmitted by adhesion is essentially

$$\sigma_{yy}(x,0) \approx \frac{2\sigma_\infty d}{\pi c}\frac{1}{\sqrt{1-x^2/c^2}} \tag{1}$$

for the particular patch occupying the interval $-c<x<c$. This general form of the distribution of transmitted stress, with relatively large stress at the edges and relatively small stress in the interior of the zone, is the principal inference to be drawn from elastic crack mechanics. The implication for the case of discretely bonded adhesion zones, when the discrete nature of the stress distribution within a patch is introduced, is that the bonds near the edge of the patch will be required to carry significantly more force than the bonds in the interior of the patch. In particular, we require that the patch transmit a total force of $2d\sigma_\infty$ over a width of $2c$ between elastic bodies, but that the distribution of discrete forces giving rise to this property be otherwise independent of the existence of adjacent patches.

Figure 2 shows a microscopic view of a representative adhesion cluster for the system in Fig. 1. The adhesion patch consists of numerous receptor-ligand bonds. The width of each bond is denoted by a and the bonds are assumed to be uniformly distributed within the cluster with spacing b. Notice that this assumption is particularly appropriate for cases in which the binding molecules on one surface have been spatially fixed [22] or have very low diffusivity; otherwise, the migration of bonds within the patch may become significant.

We emphasize that the foregoing description of load transfer across an adhesion patch with continuous bonding is included here only to establish an expectation for the force distribution among the bonds in the case of discrete bonding. No results of elastic crack analysis are actually incorporated in the model developed here, which we regard as being mechanically self-consistent.

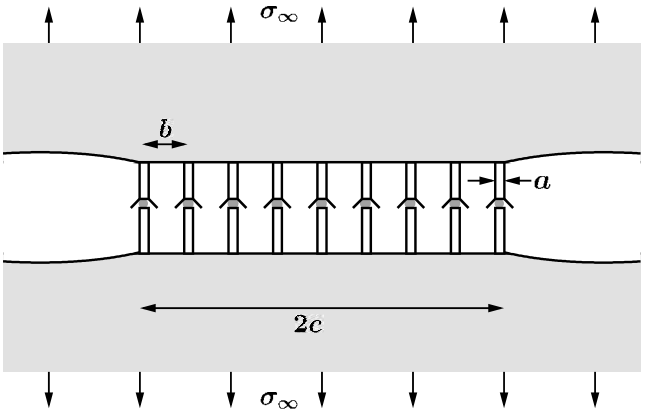

FIG. 2. Microscopic picture of one of the adhesion zones from Fig. 1, showing schematic representations of individual receptor-ligand bonds. The width of each molecule pair is denoted by a, and the spacing of bonds along the interface is denoted by b. As was indicated in Fig. 1, the overall width of the cluster is $2c$.

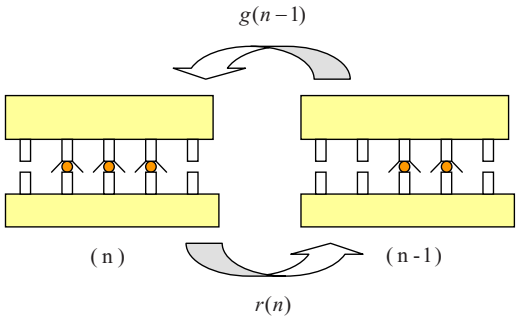

FIG. 3. (Color online) Possible transitions and transition rates between states.

III. MODEL ANALYSIS

Numerical methods were used to determine the precise nature of the discrete force distribution among any number of individual bonds. The calculation was based on the Flamant solution of plane elasticity. This solution provides the complete plane strain elastic field for the case of a concentrated normal force acting on the planar surface of an elastic solid which is otherwise stress free [23]. With this solution in hand, any distribution of stress applied in the direction normal to the surface can be constructed by means of superposition. In the present instance, we have clusters of n bonds with each such cluster forming an adhesion zone. Each individual bond has width a, and a uniform stress distribution was imposed over this interval. The net force on the bond is the resultant of the stress distributed over the interval of a. The center-to-center distance between bonds is b. The magnitude of the net force acting on each bond was determined by enforcing the condition that the surface displacement in the normal direction be uniform (the bond remains intact) and the condition that the resultant force within the cluster be equal to $2d\sigma_\infty$. To evaluate the interface strength, we first recognize that the integrity of each adhesion patch must be maintained for the interface to retain its capability to transmit force. Otherwise, the dissolution of one cluster would, in turn, result in the elevation of stress levels on the neighboring adhesion cluster edges and would thereby induce total separation of the interface.

An important aspect of the behavior of receptor-ligand bonds is that their formation or separation is generally stochastic in nature. This has been convincingly demonstrated through experimental observations by [12–14], among others. Consequently, by its nature, any bond will dissociate eventually if one waits long enough. On the other hand, any broken bond can reform if proximity is maintained. Following Bell's arguments concerning the influence of a force applied to a bond [15], the dissociation rate for bond separation when a force is present is taken to be $k_{off}=k_0e^{f/f_0}$, where k_0 is a constant, f is the magnitude of the force tending to separate the bond, and f_0 is a constant that has the same physical dimensions as f (N/m in two dimensions). For simplicity, the association rate for reestablishing a bond is assumed to have the constant value $k_{on}=1$ per unit time. This constant value implies that the time scale is set so that the reaction time for forming a single bond is one time unit. If C_b is the magnitude of energy reduction, normalized by thermal energy kT, upon formation of a single bond with no force acting on it then, from thermodynamics, k_0 is related to C_b by $k_0=e^{-C_b}$ per unit time. Typically, the reduction in energy upon the formation of a single bond is in the range between 5 and 25 kT [24]; hence, we estimate that k_0 is in the range of $10^{-4}-10^{-2}$ per unit time.

Returning to the picture of an adhesion cluster, any bond within the cluster can separate with a rate k_{off} and, conversely, any separated bond can reform with a rate k_{on}. If we consider all possibilities, then the picture becomes chaotic and analytically intractable. However, we can now take advantage of the observation from elastic crack mechanics: namely, that the edge bond is subjected to the largest force among all the bonds in the cluster. Consequently, it has the highest probability of separation; recall that the dissociation rate depends exponentially on the force. Also, consider the fact that most bonds in the interior of the cluster support relatively little force, so it is unlikely that the loads on these innermost bonds will change dramatically even if some among them are broken. Based on these observations, we further simplify the analysis by assuming that the breaking or reforming of bonds can occur only at the edges of the cluster. As a result, the accessible states of the system are represented by a single variable n, the number of intact bonds within the cluster. Hence, a potentially very complicated problem is reduced to a single-step process [25]. As shown in Fig. 3, if the system is in state n, then it can go to state $n-1$ with a rate $r(n)$ upon separation of one of the outermost bonds; conversely, the system can return to state n from $n-1$ with rate $g(n-1)$ by reforming one of the outermost bond.

Suppose that the maximum number of bonds which can be formed within a particular cluster is N. Then the value of n must lie in the range $0\leqslant n\leqslant N$. Obviously $n=0$ represents a completely separated interface. In the terminology of stochastic processes, $n=N$ is a reflecting boundary and $n=0$ is an absorbing boundary of the system [26]. Because breaking or reforming of bonds is allowed to occur only at the edges, the transition rates $r(n)$ can be calculated according to

$$r(1) = k_0 e^{F/f_0}, \quad r(n) = 2k_0 e^{F_c(n)/f_0} \quad \text{for } 1 < n \leqslant N, \quad (2)$$

where $F=2d\sigma_\infty$ is the total transmitted force and $F_c(n)$ is the force acting on either of the outermost bonds in the cluster. The magnitude of this force is calculated numerically for any state n. The factor of 2 appears in the expression for $r(n)$ because the two edges respond in the same way. Similarly, the rates $g(n)$ can be determined as

$$g(0) = g(N) = 0, \quad g(N-1) = 1, \quad g(n) = 2 \quad \text{for } 1 < n < N-1. \quad (3)$$

Notice that, in writing (3), it has been assumed that both adhesion edges can grow for any state $n<N-1$. Since we are dealing with a stochastic system with an absorbing boundary, the cluster will eventually fail—that is, will eventually reach the state $n=0$—given enough time. If the system is assumed to be in state N initially, then the average lifetime of the cluster can be calculated as [26]

$$t = \sum_{i=1}^{N} \sum_{n=i}^{N} [r(n)\Pi_{i,n-1}]^{-1}, \quad (4)$$

where $\Pi_{i,j}$ is defined as $\Pi_{i,i-1}=1$ and $\Pi_{i,j}=\frac{r(i)r(i+1)\cdots r(j)}{g(i)g(i+1)\cdots g(j)}$ for $i \leqslant j$. In order to discuss the strength of a cluster, we first need to choose a reference configuration where the "strength" is set to be zero. Here we choose $N=2$ as the reference configuration having negligible strength. This configuration has an average lifetime of

$$t_0 = \frac{1}{2k_0^2} + \frac{3}{2k_0} \quad (5)$$

when no force is acting on the cluster. For any other configuration $N>2$, we define its strength, relative to the reference configuration, as the critical load it can support such that its average lifetime is equal to t_0.

IV. RESULTS AND DISCUSSION

Choosing $b/a=2.5$, the normalized interface strength $2b\sigma_\infty^{max}/f_0C_b$ is plotted as a function of cluster size N in Fig. 4 for different k_0 values. The most striking feature illustrated in Fig. 4 is the presence of a local maximum representing an optimum cluster size for which the interface strength is maximum. We interpret this outcome as a competition between the crack mechanics aspects, which favor a smaller cluster size in order to minimize the stress concentration on the cluster edges, and stochastic considerations, which favor larger clusters in order to maintain the robustness of system. Also shown in Fig. 4 is that, as was anticipated, the normalized interface strength as defined in the way discussed above is insensitive to the particular value of k_0.

To test the validity of the model, Monte Carlo simulations of the system were carried out. In these simulations, it was assumed that any bond in the cluster, not only the outermost bonds, can separate or reform at any instant. The simulation results for $k_0=10^{-3}$ are included in Fig. 5 and these are compared with the predictions of the simple model. It is clear that the model captures almost all the important features of the behavior, such as the peak value in strength as well as the peak position (despite a small shift to the left).

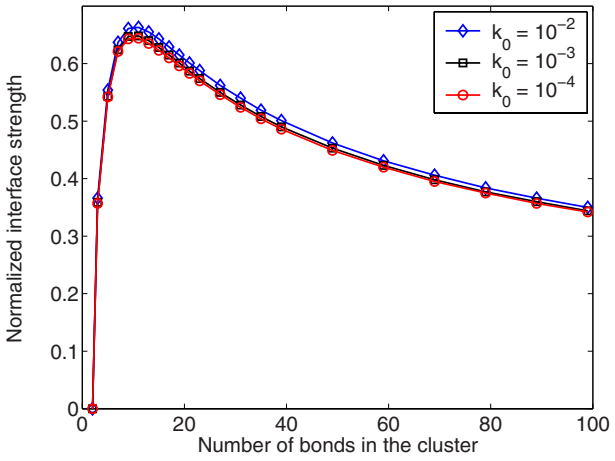

FIG. 4. (Color online) Normalized interface strength $2b\sigma_\infty^{max}/f_0C_b$ as a function of cluster size.

Although the strength varies smoothly with respect to cluster size, the cluster lifetime does not. As shown in Fig. 6, for a given load defined by $2b\sigma_\infty/f_0C_b=0.6$, the average cluster lifetime as a function of cluster size shows dramatic variation. There is a window in cluster size within which the lifetime is very large whereas, outside this window, the lifetime tends toward relatively very small values. We anticipate this has deeper implications for understanding adhesion of living cells, where a relatively uniform cluster size, usually around 1 μm in diameter [6,7], is observed among focal adhesions. It seems that this size may be directly related to the optimum cluster size as identified here, implying that the lifetime of a focal adhesion will be decreased dramatically if its size becomes larger or smaller than the critical size. In other words, it appears that a cluster is stable only within a relatively narrow size range. Indeed, as illustrated in Fig. 5, the maximum strength is achieved for a situation with about 15–25 bonds included in the cluster. Thus, the optimum cluster size is roughly 600–1000 nm if one chooses the bond spacing to be 40 nm (a typical value). This estimate is com-

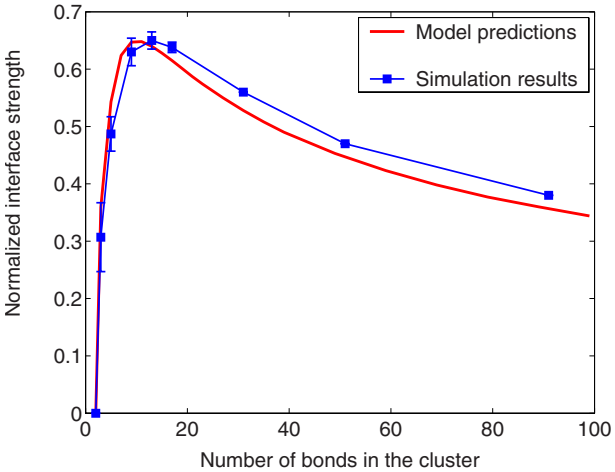

FIG. 5. (Color online) Comparison between model predictions and simulation results.

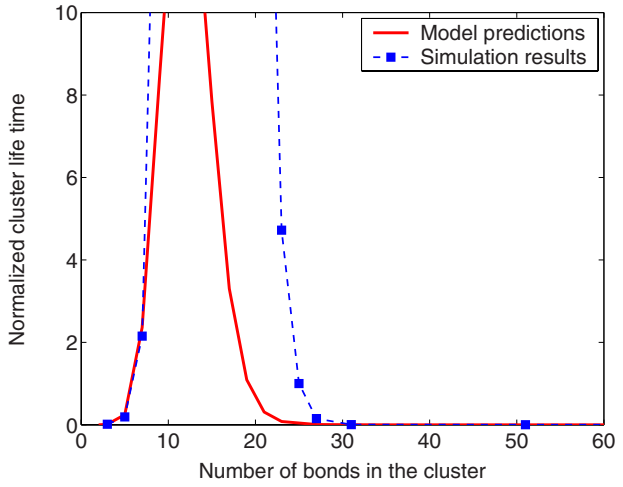

FIG. 6. (Color online) Average cluster lifetime as a function of its size.

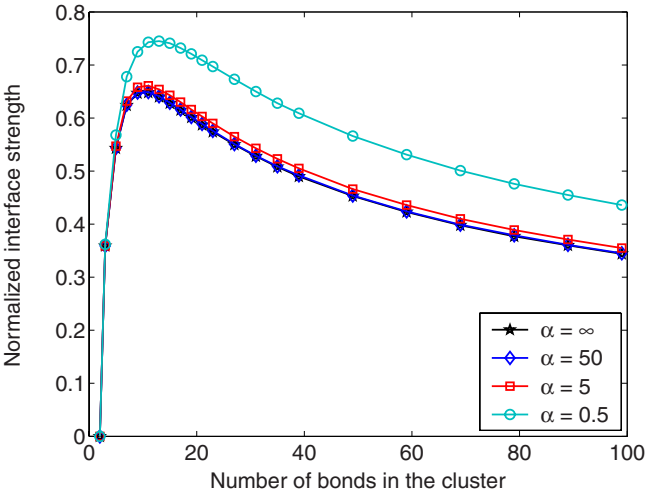

FIG. 7. (Color online) Influence of bond compliance on the interface strength.

parable to the size of focal adhesions observed in experiments [6,7]. We must point out that only the two-dimensional problem is considered here, so the conclusion that about 15 bonds per patch is optimum does not necessarily mean that there are 15 integrin molecules inside each cluster in real cell adhesions; a more realistic three-dimensional model is needed in the future to answer this question. However, we do believe that the simple model presented here does capture the essential features of the problem and the conclusion of the existence of an optimum size for adhesion patches should be pretty robust. Furthermore, the crude estimate of the number of binders in a cohesion patch being $15\times 15=225$ does seem to fall within the range of observations.

In the foregoing analysis, the deformation of the bond itself has been neglected. This presumes that a bond is much stiffer than the elastic material supporting it. To examine the influence of bond compliance, we proceed by first recalling that, from contact mechanics, the dimensionless parameter $\alpha=2C/\pi aE$ represents the ratio between the deformation of elastic continuum and the bond itself. Here, C is the spring constant of the intact bond pair, E is the modulus of the extended elastic material, and a is the width of the bond. If the Poisson ratio of the elastic material is chosen as $\nu=0.3$, a typical value, the model predictions corresponding to different values of α are shown in Fig. 7. It appears that, as long as $\alpha>5$, the results are almost indistinguishable from those obtained by neglecting the bond deformations, that is, $\alpha=\infty$. Direct Monte Carlo simulations have also verified this conclusion, but the results are not included here.

In attempting a connection to adhesion of living cells, the spring constants C for several types of bonds have been measured to be within 1–4 pN/nm [27]. The modulus of a cell as a whole is measured to be around 1–5 kPa [19], and local measurements of the bulk modulus of a cell in the vicinity of an adhesion region suggested a value of E in the range 20–40 kPa [28]. Choosing parameter values $a=12$ nm [29], $C=2$ pN/nm, and $E=2-20$ kPa, the value of α is found to be between 5 and 50. This shows that the analysis provided here may indeed have relevance to adhesion of living cells. It is interesting to point out that, if the elastic continuum is much stiffer than the bond, then a quite different scenario arises. In that case, the situation reduces to that studied by Erdmann and Schwarz [17] and Seifert [16], in which parallel bonds connecting two rigid surfaces are subjected to loading.

V. CONCLUDING REMARKS

In conclusion, the stochastic model presented here reveals the existence of an optimum adhesion cluster size for which adhesion strength is maximum. Corresponding to this strength is a maximum lifetime for the cluster. This model seems capable of explaining, from a mechanics point of view, the uniform size among mature focal adhesion regions observed in experiments. Of course, focal adhesions are required to perform numerous biological functions, so it is quite possible that strength may not be the only factor in determining the focal adhesion size. These questions need to be addressed through careful experiments and corresponding models in the future.

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