Models of dynamic extraction of lipid tethers from cell membranes

Sarah A Nowak\(^1\) and Tom Chou\(^2,3\)

\(^1\) RAND Corporation, Los Angeles, CA 90405, USA
\(^2\) Department of Biomathematics, UCLA, Los Angeles, CA 90095-1766, USA
\(^3\) Department of Mathematics, UCLA, Los Angeles, CA 90095-1555, USA

E-mail: tomchou@ucla.edu

Received 23 August 2009
Accepted for publication 26 March 2010
Published 7 May 2010
Online at stacks.iop.org/PhysBio/7/026002

Abstract

When a ligand that is bound to an integral membrane receptor is pulled, the membrane and the underlying cytoskeleton can deform before either the membrane delaminates from the cytoskeleton or the ligand detaches from the receptor. If the membrane delaminates from the cytoskeleton, it may be further extruded and form a membrane tether. We develop a phenomenological model for this process by assuming that deformations obey Hooke’s law up to a critical force at which the cell membrane locally detaches from the cytoskeleton and a membrane tether forms. We compute the probability of tether formation and show that tethers can be extruded only within an intermediate range of force loading rates and pulling velocities. The mean tether length that arises at the moment of ligand detachment is computed as are the force loading rates and pulling velocities that yield the longest tethers.

1. Introduction

Adhesion between cells plays an important role in a number of biological processes involving cell motility and cell–cell communication. Cell–cell adhesion is mediated by integral membrane proteins on the surfaces of interacting cells. Cadherins, which mediate binding between cells of the same type within a tissue, bind to themselves, while integrins, which mediate binding between different cell types bind to intercellular adhesion molecules (ICAMs) or vascular cell adhesion molecules (VCAMs) [1]. Understanding the physics of this adhesive interaction requires an understanding of both the protein–protein bond as well as the cell’s mechanical response when these bonds become stressed. If forces act to pull the two cells apart, either of the cells’ cytoskeletons and plasma membranes may deform. Under certain conditions, the lipid membranes can delaminate from the underlying cytoskeleton and be pulled into long tethers. At any time during this process, the bonds holding the two cells together may also break, arresting tether extraction.

A specific biological process in which bond dissociation and membrane deformation must be considered simultaneously is leukocyte extravasation, which is part of the process by which leukocytes are recruited to inflamed or infected tissue. Endothelial cells that make up blood vessels preferentially express cellular adhesion molecules, including selectins, near wounded tissue. Leukocytes circulating in the blood can then bind to the endothelial cells via their own cell surface proteins. Bonds between the leukocytes and the endothelial tissue are transiently made and broken as a shear flow in the blood vessel pushes the leukocyte, rolling it across the endothelial layer [2]. The rolling leukocytes contain microvilli that are enriched in adhesion molecules that preferentially attach to endothelial cells. During rolling, these microvilli tethers can extend under the hydrodynamic shear force of blood flow in the vessel [3]. At the same time, forces imposed on the endothelial membrane via the adhesion molecule can cause the endothelial membrane to form a tether [4]. Tether formation and extension of microvilli can decrease the force that the adhesive bond feels from the shear blood flow.

Both the physics of cell membrane deformation and the mechanics of ligand–receptor bonds have been studied extensively. Micropipette, atomic force microscope (AFM), optical trap and magnetic bead techniques have been used to pull membrane tethers from cells and probe the properties of the cell membrane [5]. The diameter of the extracted tether depends on the membrane surface tension and bending rigidity
and, if the tether is being extended at a constant velocity, the membrane viscosity [6, 7]. Therefore, these quantities can be inferred from tether pulling or poking experiments [8]. Theoretically, Euler–Lagrange methods have been used to compute equilibrium tether shapes and the force–extension curve for a tether pulled quasi-statically from a lipid vesicle [9, 10]. These theoretical models of pure lipid bilayers show only a ∼10% overshoot, or barrier, in the force–extension curve before a tether is extracted from an asymptotically flat membrane [9, 10]; some experiments show a significant force barrier to tether formation [11–13]. These large force barriers to membrane tether formation arise in living cells and are attributed to membrane adhesion to the underlying actin cytoskeleton [11, 14]. When tethers are pulled from giant artificial vesicles, the size of the force barrier can increase only when the area on which the pulling force is exerted is increased [12]. For smaller vesicles, area and volume constraints may also influence the tether force–extension relationship [15]. These may arise from nonlocal terms in the functional describing the lipid membrane energetics. For example, area–difference elasticity can give rise to a restoring forces that continually increase as tether length increases [16, 17]. Such nonlocal effects will be important only when the tether comprises an appreciable fraction of the total membrane area. Force curves that do not saturate at long tether extensions can also arise when part of the membrane reservoir adheres to a substrate [13]. The relative importance of nonequilibrium forces arising from the viscosity of both the membrane lipids and surrounding solution has also been estimated [18].

During tether pulling experiments, and in their corresponding theoretical models, the molecular bond attaching the pulling device to the membrane is assumed to always remain intact. However, a typical ligand–receptor bond used to connect the pulling device to the lipid membrane (and possibly the underlying cytoskeleton) can rupture upon pulling. Although the details of a bond’s energy landscape can be probed using dynamic force spectroscopy [19–22], one can usually assume that bond rupturing is dominated by a single activation barrier that can be lowered by an externally applied pulling force. Most AFM studies of molecular strength are performed on proteins that have been isolated from cells. However, recent studies have probed bond strengths between proteins still embedded in a live cell membrane [23, 24]. While using live cells has the advantage that the post-translational modifications of membrane proteins are preserved, the mechanical deformation of the cell’s cytoskeleton and membrane must also be taken into account. To model this system, a viscoelastic Kelvin model was used to fit experimental measurements of the force–extension relationship to determine effective cellular adhesion [24].

In this paper, we develop a dynamic model that incorporates phenomenological theories of membrane and cytoskeleton deformation, tether extraction and the kinetics of ligand–receptor detachment. In contrast to an equilibrium model determining tether extraction and detachment from an adhered vesicle [15], we find relationships that define when tether extraction is likely, and the typical length of the tether pulled before the ligand–receptor bond ruptures.

In the next section, we motivate a simple mechanical model using phenomenological forms for the force–extension relationship of the membrane. Given the large bending energies of lipid bilayer membranes, and the relatively strong attachment of membranes to cytoskeleton, we will neglect thermal fluctuations of the membrane, but implicitly include thermally-driven ligand–receptor bond dissociation. Dynamical equations are written for two commonly employed experimental protocols, a linear force loading rate and a constant bond pulling speed. In section 3, we compute the probability of tether formation and plot universal curves that delineate regimes where tethers are likely to form. Mean tether lengths are also plotted for both pulling protocols.

2. Mathematical model

Consider the system depicted in figure 1. A ligand is bound to an integral membrane protein, which may also be directly associated with the cytoskeleton. The ligand may be attached to a pulling device via a cantilever spring and is pulled with either a fixed speed, or a force that increases linearly in time.

As the ligand is pulled, the cytoskeleton first deforms, and eventually can detach from the membrane. At this point, the lipid membrane may flow into a tether. At any point during the cytoskeletal or membrane deformation, before or after membrane–cytoskeleton delamination and tether formation, the ligand affixed to the pulling device may detach from the membrane receptor protein.
2.1. Membrane mechanics

We first consider the response of the membrane–cytoskeleton system to an externally applied pulling force $F_p(t)$. The rate at which the receptor–ligand complex moves will be described by

$$\frac{dx(t)}{dt} = -\xi \left[ \frac{\partial U(x)}{\partial x} \right]_{x=x(t)} - F_p(t)$$

(1)

where $\xi$ is a mobility that is inversely related to the viscosity of the membrane lipid [18]. In general, $\xi$ depends on the configuration of the system defined predominantly by $x(t)$; however, we neglect the details of this dependence and assume it to be constant.

The term $U(x)$ represents the energetic cost associated with deforming the cell membrane and underlying cytoskeleton when the receptor is displaced by a distance $x$ normal to the flat membrane. This phenomenological energy can be derived from a detailed consideration of the membrane–cytoskeletal mechanics. For simplicity, we will assume the membrane mechanics are governed by a Helfrich free energy [25] that includes a bilayer bending rigidity and an effective thermally derived entropic membrane tension. We will assume that the membrane reservoir is large enough such that an extruded tether negligibly depletes the reservoir. Hence, global contributions to the membrane energetics, such as area–difference elasticity, can be neglected.

Experiments in which tethers were pulled from live cells found a significant force barrier to tether formation [11, 14]. While a smaller force barrier can also arise in pure lipid membranes [9], we will assume that the plasma membrane is attached to an underlying cytoskeleton (with anchoring molecules), which we model as a linear elastic material provided the deformation is small. The receptor that binds the ligand that is attached to the pulling device can also be a transmembrane receptor that is directly attached to the cytoskeleton. As the membrane is initially pulled, the cytoskeleton will elastically deform as a Hookean spring. The receptor or anchoring molecules will break at a deformation $x_0$, and the lipid membrane will be drawn into a tether. This occurs at a critical delamination force $F_c$. Thus, for displacements $x < x_0$, where the Hookean approximation for the membrane–cytoskeleton assembly is valid, the membrane–cytoskeleton carries an effective spring constant $F_c/x_0$, where $F_c$ is the critical delamination force. Experimentally, the cytoskeleton typically detaches from the membrane where the filament-free tether forms [26, 27]; therefore, we can assume a linear force–extension relationship of the form

$$\frac{\partial U}{\partial x} = \frac{x}{x_0} F_c, \quad x < x_0.$$

(2)

At a displacement $x = x_0$, the membrane delaminates from the cytoskeleton and a lipid membrane tether forms. Under the infinite reservoir and simple Helfrich free-energy assumption, the tether can elongate indefinitely under a constant force $\frac{\partial U}{\partial x} = F_0$ that is intrinsic to the lipid tether and is determined by the membrane bending rigidity $\kappa$ and entropic surface tension $\sigma$ [9, 10];

$$F_0 = 2\pi \sqrt{2k_\sigma \sigma}.$$  

The phenomenological membrane force–displacement relationship for both attached (solid curve) and free (dashed curve) membranes is shown in figure 2. The barrier $F_c$ to tether formation is larger for receptors or membranes that are attached to the underlying cytoskeleton, than for the free lipid membrane case.

In order to close the equations of our basic model, we must specify $F_p(t)$. Henceforth, we will consider two cases typically realized in experiments: a linearly increasing (in time) pulling force and a fixed pulling speed.

2.2. Linear force ramp

For a force linearly increasing in time, $F_p(t) = \Gamma t$, where $\Gamma$ is the rate with which the force increases. Equation (1) becomes

$$\frac{dx(t)}{dt} = -\xi \left[ \frac{\partial U(x)}{\partial x} - \Gamma t \right].$$

(3)

Upon defining the time $t_0$ at which the membrane delaminates from the cytoskeleton provided the ligand–receptor bond has not yet ruptured by $x(t = t_0) \equiv x_0$, we have $\frac{\partial U}{\partial x} = (x/x_0)F_c$ for $t \leq t_0$, and $\frac{\partial U}{\partial x} = F_0$ for $t > t_0$. Thus, equation (3) is solved by

$$x(t < t_0) = x_0 \frac{\Gamma}{F_c} - \frac{\Gamma x_0^2}{\xi F_c^2} \left(1 - e^{-\xi F_c/t_0}\right),$$

(4)

and

$$x(t > t_0) = x_0 - \xi F_0(t - t_0) + \frac{\Gamma}{2} \left(t^2 - t_0^2\right),$$

(5)

where the delamination time $t_0$ is determined from the solution to

$$\frac{\Gamma}{F_c} t_0 = \frac{\Gamma x_0}{\xi F_c} \left(1 - e^{-\xi F_c/t_0}\right) = 1.$$  

(6)
2.3. Constant pulling speed

In the case of constant pulling speed, we must include the dynamics of the device deformation $\ell(t)$. Since the velocity of the pulling device is fixed, we note that the total displacement $z(t) = x(t) + \ell(t)$ obeys

$$\frac{dz}{dt} = \frac{dx}{dt} + \frac{d\ell}{dt} = V. \quad (7)$$

This equation holds only when the ligand is attached to the membrane-bound receptor. Let us assume that the pulling device has an internal response that is modeled by a simple spring so that the force $F_p(t)$ that the spring exerts on the ligand is

$$F_p(t) = K \ell(t), \quad (8)$$

where $K$ is the spring constant of the pulling device. Since the pulling force is proportional to $\ell(t)$, it will ultimately depend on the pulling rate $V$ and the physical properties of the pulling device (represented by an elastic cantilever in figure 1) and cell membrane through equation (7). Upon integrating equation (7) and using the initial conditions $x(t=0) = \ell(t=0) = 0$, we find

$$x(t) = VT - \ell(t). \quad (9)$$

Substituting equations (9) and (8) into equation (1), and expressing the dynamics in terms of the device deformation, we find a closed equation for $\ell(t)$:

$$\frac{d\ell}{dt} = V + \xi \left[ \frac{\partial U(x)}{\partial x} \bigg|_{x=VT-\ell(t)} - K \ell(t) \right]. \quad (10)$$

This equation is solved by

$$\ell(t \leq t_0) = \frac{V K x_0^2}{\xi (F_c + K x_0)} (1 - e^{-\xi(K+K_{fr}/x_0)t}) + \frac{F_c V T}{F_c + K x_0} \quad (11)$$

for $t < t_0$, and

$$\ell(t > t_0) = \frac{V + \xi F_0}{\xi K} (1 - e^{-\xi(K+K_{fr}/x_0)t} + \ell(t_0)) e^{-\xi(K+K_{fr}/x_0)t} \quad (12)$$

for $t > t_0$ (when $\partial U/\partial x = F_0$). Here, the time $t_0$ at which tether formation occurs is found by evaluating equation (9) at time $t = t_0$, $x(t_0) = x_0 = VT - \ell(t_0)$, yielding an implicit equation for $t_0$:

$$V t_0 - x_0 = F_c V T_0 \quad (F_c + K x_0) + \frac{V K x_0^2}{\xi (F_c + K x_0)} (1 - e^{-\xi(K+K_{fr}/x_0)t_0}). \quad (13)$$

After evaluating $t_0$ numerically, $\ell(t)$ is found in terms of the $K, V, \xi, F_c, F_0$ and $x_0$, and the membrane displacement can be found using equation (9).

2.4. Ligand–receptor dissociation

The dynamics described above for the membrane and pulling device deformations assume that the pulling device remains attached to the membrane through an unbroken ligand–receptor bond. Since all external forces are transduced through the ligand–receptor bond, the pulling force $F_p(t)$ on the membrane (cf equation (1)) vanishes once the ligand–receptor bond ruptures. However, the probability of ligand–receptor bond dissociation itself depends on the applied force $F_p(t)$. We can model the breaking of the ligand–receptor bond by a Poisson process and define a ligand–receptor bond survival probability $Q(t)$ that obeys

$$\frac{dQ(t)}{dt} = -k_r(t) Q(t). \quad (14)$$

where $k_r(t)$ is the force-dependent rupture (or dissociation) rate of the ligand from the receptor. We assume that $k_r(t)$ takes a simple Arrhenius form [28]:

$$k_r(t) = k_0 e^{F_0(t)d/k_B T}, \quad (15)$$

where $d$ is the length of the ligand–receptor bond and $k_BT$ is the thermal energy. The solution to equation (14) is explicitly

$$Q(t) = \exp \left[ -k_0 \int_0^t e^{F_0(\tau)d/k_B T} d\tau \right]. \quad (16)$$

More complex models of dynamics bond rupturing can be derived [29, 30]. Here, for simplicity, molecular details such as the thermally-induced bond-breaking attempt frequency and the intrinsic free energy of the unstrained ligand–receptor bond are subsumed in the effective rate parameter $k_0$.

Since $w(t) = -dQ(t)/dt = k_r(t) Q(t)$ is the bond rupture time distribution, the mean membrane displacement at the time of ligand–receptor rupture (the mean maximum displacement) is given by

$$\langle x^* \rangle = \int_0^\infty w(t) x(t) \ dt = \int_0^\infty k_r(t) Q(t) x(t) \ dt. \quad (17)$$

In our subsequent analysis, we will combine bond rupturing statistics with membrane tether dynamics and explore, as a function of the physical parameters, the probability of tether formation and the length of pulled tethers they should form. To be concrete, we will use typical parameter values (listed in table 1) found from the relevant literature to guide our analysis.

3. Results

Here, we compute the dynamics of tether formation and ligand–receptor bond rupturing under both linear force loading and constant pulling velocity protocols.

### Table 1. Typical parameter values.

| Parameter | Range of values | Reference |
|-----------|----------------|-----------|
| $d$ | 0.8–1.0 nm | [31] (Streptaviden-HABA) |
| $\xi$ | $\sim$1 $\mu$m (pN s)$^{-1}$ | [18] |
| $x_0$ | $\sim$1–4 $\mu$m | [11] |
| $F_0$ | 3–380 pN | Calculated$^a$ |
| $F_c$ | 100–380 pN | [27] (Red blood cells) |
| 1–100 pN | [32] (Epithelial cells) |
| $K$ | 8–11 pN $\mu$m$^{-1}$ | [11] |
| $k_0$ | $10^{-10}$–10 s$^{-1}$ | [33] |
| $\nu$ | $\sim$3 $\mu$m s$^{-1}$ | [11] |
| $\gamma$ | 10 pN s$^{-1}$ | [11] |

$^a$ $F_0 = 2\sqrt{2\pi} \kappa \sigma$ where $\kappa$ is the membrane bending rigidity and $\sigma$ is the effective membrane surface tension [9]. We assumed $\sigma = 3–1200$ pN $\mu$m$^{-1}$ [34] and $\kappa = 10–20$ $k_BT$ [35].
dependence is on the delamination force $F_c$ which sets the delamination $t_0$ (cf equation (6)) in the expression $P_T \equiv Q(t_0)$. Once the tether is formed, the free tether restoring force $F_0$ is irrelevant. The values $\Gamma_+$ and $\Gamma_-$ are defined by $P_T(\Gamma_{\pm}) = 1/2$ and define the window of loading rates within which tether formation is likely.

For quantitative evaluation of $\Gamma_{\pm}$ and their dependences on the other system parameters ($F_{\text{max}}$, $k_BT$, $x_0$, and $\xi$), it is convenient to define dimensionless parameters according to $\gamma \equiv \xi \Gamma/k_{\text{BT}} x_0$, $\alpha \equiv k_{\text{BT}} d/(\xi k_B T)$, and $f_c = \xi F_c/k_{\text{BT}} x_0$, (19) and find parameter regimes within which $P_T > 1/2$. Using equation (18), the phase boundaries for tether formation are determined from the implicit solution to

$$\exp \left[ -\frac{1}{\alpha \gamma}(e^{\alpha \gamma t_0(y/f_c)} - 1) \right] = \frac{1}{2}. \quad (20)$$

Figure 3(b) shows curves in dimensionless parameter space ($f_c$ and $\gamma$) below which $P_T > 1/2$. Asymptotic analysis of the condition $P_T = 1/2$ shows that for sufficiently large $\gamma$, tether formation will always be suppressed. Additionally, as the dimensionless ligand–receptor dissociation rate $\alpha$ increases, the regime for tether formation shrinks.

Conditions for tether formation can be further refined by computing universal parameter curves that define regimes for which $P_T$ can never be greater than 1/2. As a function of the intrinsic ligand–receptor dissociation rate, there is a band of pulling rates outside of which $P_T$ is always less than one-half, even when tether extraction is barrierless ($f_c = 0$). Figure 3(c) shows $\gamma_{\text{max}}$ and $\gamma_{\text{min}}$, the minimum and maximum dimensionless ramp rates at which $P_T = 1/2$ when $f_c = 0$. These delimiting loading rates are roots of equation (20).

For small $\alpha$, the lower root $\gamma_{\text{max}} \approx 2(1/\ln^2 2 + O(\alpha))$. Also plotted in figure 3(c) is $f_{c\text{max}}(\alpha)$, the maximum membrane–cytoskeleton delamination force that can give rise to $P_T > 1/2$, for any ramp rate. One is unlikely to pull tethers from membranes that require more force than $f_{c\text{max}}$ to delaminate from the cytoskeleton. Moreover, there is a critical $\alpha_{\text{max}} \approx 0.22444$ above which $P_T = 1/2$ cannot be reached, independent of $f_c$ or $\gamma$.

Finally, we plot in figure 4(a) the expected dimensionless maximum tether length $(X^*) = \langle x^* \rangle x_0$ found from equation (17), as a function of the dimensionless force loading rate $\gamma$. Not only does the mean tether length decrease with increasing critical delamination force $f_c$, as shown in figure 4(a), but the value of $\gamma$ at which $(X^*)$ is maximized decreases as $f_c$ is raised. Since tether extraction is a competition between the rate of climb against the restoring force $F_c x/ x_0$ and ligand–receptor dissociation, as $f_c$ is increased, higher ramp rates are required to cross the delamination barrier faster, relative to the bond rupturing. Although the free membrane restoring force $F_0 = \xi F_0/(k_{\text{BT}} x_0)$ does not come into play in the tether formation probability, it does influence the mean length of

Figure 3. (a) Tether formation probability $P_T \equiv Q(t_0)$ as a function of force ramp rate $\Gamma$. Note that results for the constant force ramp protocol are independent of the free tether restoring force $F_0$. $\Gamma_{\pm}$ denote the linear loading rates at which the tether formation probability $P_T = 1/2$. Applied loading rates $\Gamma_+ < \Gamma < \Gamma_-$ are likely to lead to tether formation. For $F_c = 20$ pN, $\Gamma_{\pm} \approx 7.2$ pN s$^{-1}$ and $\Gamma_{\pm} \approx 435$ pN s$^{-1}$. Other parameters used were $k_o = 0.01$ s$^{-1}$, $d = 1$ nm, $x_o = 1$ nm and $\xi = 1$ nm (pNs)$^{-1}$. (b) Dimensionless parameter regimes in which $P_T > 1/2$. The regions of parameter space below each curve are associated with $P_T > 1/2$, where tether formation is likely. The smaller the dimensionless bond dissociation rate $\alpha = k_o x_0 d/(\xi k_B T)$, the wider the region of dimensionless loading rates $\gamma = \xi \Gamma k_{\text{BT}} x_0$ leading to tether formation. The maximum and minimum pulling rates $\gamma_{\text{min}} \equiv \gamma_r(f_c = 0)$ and $\gamma_{\text{max}} \equiv \gamma_r(f_c = 0)$ are indicated for the $\alpha = 0.006$ curve, while the maximal dimensionless delamination force $f_{c\text{max}}$ is shown for $\alpha = 0.0004$. (c) The minimum and maximum force ramps, and the maxima delamination force as functions of $\alpha$. For $\gamma < \gamma_{\text{min}}$, $\gamma > \gamma_{\text{max}}$ or $f_c > f_{c\text{max}}$, tethers always have less than 50% chance of forming.

3.1. Linear force ramp

When $F_0(t) = \Gamma t$, the ligand–receptor survival probability defined by equation (16) is explicitly

$$Q(t) = \exp \left[-\frac{k_o k_B T}{\Gamma d} (e^{\Gamma t/d} - 1) \right]. \quad (18)$$

The bond survival probability, evaluated at the time of tether formation $t_0$ (found numerically from equation (6)), $Q(t_0) = P_T$, determines the likelihood that a tether is extracted, and is plotted in figure 3(a) as a function of force loading rate $\Gamma$. Note that $P_T$ first increases with the force loading rate $\Gamma$, before decreasing again at very high loading rates. Large critical delamination forces $F_c$, increase the probability that ligand–receptor bonds detach before membrane–cytoskeleton delamination occurs. Membrane–cytoskeleton combinations that have weaker delamination forces $F_c$ yield a larger range of force loading rates that lead to tether formation. Moreover, since the pulling force is specified, $Q(t)$ is independent of the free tether restoring force $F_0$ (cf equation (18)).
lipid tether that is extruded before the ligand detaches from the membrane-bound receptor. Note that in the constant loading rate protocol, the force is specified and all results are independent of the pulling device rigidity $K$.

### 3.2. Constant pulling speed

Now consider a constant pulling speed protocol. The force felt by the membrane in this ensemble will depend on the pulling device rigidity $K$. The bond survival probability is computed from

$$Q(t) = \exp \left( -k_0 \int_0^t kT/\kappa d\ell' dr' \right), \quad (22)$$

where $\ell(t)$ is given by equations (11) and (12). Initially, while the membrane and cytoskeleton are attached to each other, and constant speed pulling is applied, the ligand–receptor bond survival probability $Q(t)$ first decreases rapidly. After delamination, the forces on the ligand–receptor are fixed, arising only from $F_0$ and the viscous drag $\xi^{-1}$. The subsequent decay of $Q(t)$ arises from a slower, single exponential.

Figure 5(a) shows the corresponding $P_T$ as a function of pulling speed $V$, and as in the force ramp case, reveals an optimal pulling speed that maximizes the likelihood of pulling a tether. In the $V \rightarrow 0$ limit $x(t) \approx 0$ (from equations (9) and (12)), and we expect $P_T \rightarrow 0$ because when the ligand–receptor bond detaches, the membrane has not been sufficiently deformed. In the fast pulling limit, the detachment rate increases quickly and ligands detach at very short times $t$ such that tether formation cannot occur. Upon defining $P_T(V_{\pm}) = 1/2$, pulling speeds $V_- < V < V_+$ are likely to result in tethers.

To explore how $V_{\pm}$ depends on other system parameters, we employ the same dimensionless parameters defined in equations (19), along with a dimensionless pulling speed and pulling device stiffness,

$$v \equiv \frac{V}{k_0x_0} \quad \text{and} \quad \mu \equiv \frac{K\xi}{k_0}. \quad (23)$$

Figure 5(b) shows, for $\mu = 1000$ and various values of $\alpha$, the boundaries below which tether formation is likely.

Figure 5(c) shows the phase boundaries for fixed $\alpha = 0.002$ and various pulling device stiffnesses $\mu$. Note that softer pulling devices suppress tether formation at low speeds since the forces are not immediately felt by the membrane, allowing the ligand more time to detach. However, softer pulling devices greatly enhance tether formation at large pull speeds because the accelerated delamination more than compensates for the drag-mediated acceleration of ligand–receptor dissociation.

Note that analogous to the linear force ramp protocol, for particular $\alpha$ and $\mu$, there is a maximum and minimum pulling speed ($v_{\text{min}}^\text{max}$ and $v_{\text{max}}^\text{min}$) beyond which tether formation is unlikely, even when the delamination force vanishes (figure 6(a)). Conversely, there is a maximum delamination force $f^\text{max}$ above which no pulling speed will result in likely tether formation (figure 6(b)).

Finally, consider the mean receptor displacement at the moment of ligand detachment, $(X'(t)) \equiv \langle x(t) \rangle /x_0$, found from equation (17) with $X(t) = vT - \ell(t)/x_0$, and the appropriate $Q(t)$ and $k_0(t)$. The same arguments that explain the non-monotonic behavior of $P_T$ as a function of $V$ apply here, and the mean dimensionless receptor displacement at the moment of ligand detachment, $(X'(t))$, is a non-monotonic function of $V$ (figure 7). When the pulling velocity is small, the receptor moves slowly and the term $x(t)$ in equation (17) will be small, rendering integral in equation (17) small. On the other hand, when $V$ is sufficiently large, the viscosity $\xi^{-1}$ allows a large force to be reached, accelerating ligand–receptor
acts on the ligand also increases linearly in time: In the extremely soft pulling device limit, the elastic pulling a high constant force loading rate of relationship before delamination, this protocol is equivalent to $v_c$ slightly larger than the $\alpha$ is a non-monotonic function of the dimensionless pulling velocity, $\alpha(b)$.

Figure 7. (a) The mean system length at ligand detachment, $\langle X^* \rangle$, is a non-monotonic function of the dimensionless pulling velocity, $v$, and increases with decreasing $f_c$. The qualitative dependence of $\langle X^* \rangle$ on $f_c$ is similar to that shown in figure 4. (b) The mean maximum tether length as a function of $v$ for various dimensionless pulling device stiffnesses $\mu$.

bond rupturing. Thus, $Q(t)$ quickly decreases and the mean receptor displacement when the ligand detaches, $\langle x^* \rangle$, will be small.

Both constant force load rate and constant pulling speed protocols give qualitatively similar tether extraction probabilities and maximum delamination forces $f_c^{\text{max}}$. This is not surprising since the two protocols are physically equivalent in both the infinitely stiff and infinitely soft pulling device limits, prior to delamination. In the large $\mu \ll K$ limit, a constant pulling speed forces the ligand to have the trajectory $x(t) = Vt$. Because we assume a linear force–extension relationship before delamination, this protocol is equivalent to a high constant force loading rate of $\Gamma \approx KV$ (or $\gamma \approx \mu V$). In the extremely soft pulling device limit, the elastic pulling device absorbs most of the extension and the force at which it acts on the ligand also increases linearly in time: $\Gamma \approx F_c V/X_0$ (or $\gamma \approx v_c f_c$).

However, we do find a qualitative difference in the mean tether length extracted, due to the difference in the post-delamination forces between the two protocols. As functions of load rate and pulling speed, the maximum mean tether lengths attainable via linear force ramp are typically less than half of those achieved through constant pulling speed, all else being equal. This feature can be understood by considering how the receptor displacement, $x(t)$, and the ligand dissociation rate, $k_l(t)$, depend on time in each case. When the pulling speed is constant, after the tether forms, $x(t)$ increases linearly in time, and $k_l(t)$ is constant. When we apply a force ramp to the system, $x(t)$ increases quadratically in time once tether formation occurs. This would seem to imply longer tethers under the force ramp protocol; however, in this case, the dissociation rate $k_l(t)$ also increases exponentially in time. Thus, ligand detachment is much faster in the force ramp case, resulting in shorter observed mean tether lengths.

4. Summary and conclusions

We modeled membrane–cytoskeleton delamination in series with a ligand–receptor bond and a deformable pulling device and determined the parameter regimes within which lipid tether extrusion is likely. Results from our model can be directly used to propose and analyze experiments in which cell or lipid vesicle membranes are pulled by a breakable bond. For example, in [11], tethers are pulled from endothelial cells when large force barriers are overcome, but detachment of the pulling device from the tether is not considered. Performing such experiments with breakable ligand–receptor bonds would provide the necessary data with which to test our predictions on the likelihood of tether formation and on the differences between fixed load rate and pulling speed protocols.

For both linear force ramp and constant pulling speed protocols, we find a wide window of ramp rates and pulling speeds that likely lead to tether extraction. However, we also find critical values of a dimensionless membrane–cytoskeleton delamination force, and a dimensionless spontaneous ligand–receptor dissociation rate beyond which tether formation is unlikely, regardless of all other parameters. We assumed in all of our analysis that the tether force–extension curve can be derived from local interactions with a Helfrich free-energy model. Finite-size membrane reservoirs and nonlocal energies such as area–difference elasticity would give rise to increasing forces as the tether is extended, thereby increasing the probability of ligand–receptor dissociation, and decreasing expected tether lengths $\langle X^* \rangle$.

Both linear force ramp and constant pulling speed protocols yield intermediate tether formation regimes, with a specific pulling speed $v$ and specific linear ramp rate $\gamma$ that maximizes the mean tether length $\langle X^* \rangle$ in the respective protocol. However, they present different tether dynamics after delamination leading to different expected tether lengths $\langle x^* \rangle$. Using both protocols, and our results, it may be possible to characterize membrane–cytoskeleton properties, provided sufficient information about the ligand–receptor binding energy and pulling device response are known. In general, such inverse problems are very ill-posed, but restricting the force–extension relationship to simple forms as we have done, one may be able to use the onset of tether
formation as a way to estimate force parameters (such as $F_0$, $F_c$ and $x_0$).

While we have framed our analysis in terms of AFM experiments in which the strength of a ligand–receptor bond is probed while the receptor is in the membrane of a live cell, our basic model is relevant to leukocyte rolling as well. In leukocyte rolling, a bond between protein on a leukocyte microvilli and a protein in the membrane of an endothelial cell becomes stressed. Because the microvilli act like Hookean microvilli and a protein in the membrane of an endothelial cell has been observed [4], our analysis is directly applicable to this system, with the cantilever replaced with a microvilli.

Finally, we note that we have treated the ligand–receptor bond rupturing as a stochastic Poisson process, while the deformation of membrane and cytoskeleton was considered deterministic. This approximation is good as long as $x_0 \gg d$. However, if the experiment is repeated, each region of membrane may have highly variable attachments to the cytoskeleton. In this case, a distribution of delamination forces $F_c$ should be considered. Another source of stochasticity may arise when multiple adhesion points are being pulled, possibly leading to multiple tethers [36]. If the entire system is treated as a single, effective tether, the force–extension of this super-tether will rely on the statistics of how many individual tethers are still attached during the dynamics.

Acknowledgments

This work was supported by the NSF through grant no DMS-0349195 and by the NIH through grant no K25 AI058672.

References

[1] Lodish H, Berk A, Matsudaira P, Kaiser C A, Krieger M, Scott M P, Zipursky S L and Darnell J 2003 Molecular Cell Biology (San Francisco, CA: Freeman)

[2] Korn C B and Schwarz U S 2008 Dynamic states of cells adhering in shear flow: from slipping to rolling Phys. Rev. E 77 041904

[3] Shao J Y, Ting-Beall H P and Hochmuth R M 1998 Static and dynamic lengths of neutrophil microvilli Proc. Natl Acad. Sci. USA 95 6797–802

[4] Girdhar G and Shao J Y 2007 Simultaneous tether extraction from endothelial cells and leukocytes: observation, mechanics, and significance Biophys. J. 93 4041–52

[5] Schumacher K, Popel A S, Anvari B, Brownell W E and Spector A A 2009 Computational analysis of the tether-pulling experiment to probe plasma membrane–cytoskeleton interaction in cells Phys. Rev. E 80 041905

[6] Hochmuth R M and Evans E 1982 Extensional flow of erythrocyte membrane from cell body to elastic tether Biophys. J. 39 71–81

[7] Dai J and Sheetz M P 1995 Mechanical properties of neuronal growth cone membranes studied by tether formation with laser optical tweezers Biophys. J. 68 988–96

[8] Fygenson D, Marko J F and Libchaber A 1997 Mechanics of microtubule-based membrane extension Phys. Rev. Lett. 79 4407–500

[9] Powers T R, Huber G and Goldstein R E 2002 Fluid-membrane tethers: minimal surfaces and elastic boundary layers Phys. Rev. E 65 041901

[10] Derényi I, Julicher F and Prost J 2002 Formation and interaction of membrane tubes Phys. Rev. Lett. 88 238101

[11] Sun M, Graham J S, Hegedus B, Marga F, Zhang Y, Forgacs G and Grandbois M 2005 Multiple membrane tethers probed by atomic force microscopy Biophys. J. 89 4320–9

[12] Koster G, Cacciuto A, Deręzy I, Frenkel D and Dogterom M 2005 Force barriers for membrane tube formation Phys. Rev. Lett. 94 068101

[13] Cuveller D, Chiaruttini N, Bassereau P and Nassy P 2005 Pulling long tubes from firmly adhered vesicles Europhys. Lett. 71 1015–21

[14] Benoit M, Gabriel D, Grerisch G and Gaub H E 2000 Discrete interactions in cell adhesion measured by single-molecule force spectroscopy Nature Cell Biol. 2 313–7

[15] Smith A-S, Sackmann E and Seifert U 2004 Pulling tethers from adhered vesicles Phys. Rev. Lett. 92 208101

[16] Glassinger E and Raphael R M 2006 Influence of thermally driven surface inductions on tethers formed from bilayer membranes Biophys. J. 91 619–25

[17] Raphael R M and Waugh R E 1996 Accelerated interleaflet transport of phosphotidylcholine molecules in membranes under deformation Biophys. J. 71 1374–88

[18] Hochmuth F M, Shao J Y, Dai J and Sheetz M P 1996 Deformation and flow of membrane into tethers extracted from neuronal growth cones Biophys. J. 70 358–69

[19] Merkel R, Nassy P, Leung A, Ritchie K and Evans E 1999 Energy landscapes of receptor–ligand bonds explored with dynamic force spectroscopy Nature 397 50–3

[20] Evans E 2001 Probing the relation between force-lifetime and chemistry in single molecular bonds Annu. Rev. Biophys. Biomol. Struct. 30 105–28

[21] Heymann B and Grubmüller H 2000 Dynamic force spectroscopy of molecular adhesion bonds Phys. Rev. Lett. 84 6126–9

[22] Duško O K, Filippov A E, Klafter J and Urbakh M 2003 Beyond the conventional description of dynamic force spectroscopy of adhesion bonds Proc. Natl Acad. Sci. USA 100 11378–81

[23] Hanley W, McCarty O, Jadhav S, Tseng Y, Wirtz D and Konstantopoulos K 2003 Single molecule characterization of p-selectin/ligand binding J. Biol. Chem. 278 10556–61

[24] Schmitz J, Benoit M and Gottschalk K E 2008 The viscoelasticity of membrane tethers and its importance for cell adhesion Biophys. J. 95 1448–59

[25] Miao L, Seifert U, Wortis M and Döbereiner H G 1994 Budding transitions of fluid-bilayer vesicles: the effect of area–difference elasticity Phys. Rev. E 49 5389–407

[26] Afri R and Ikai A 2006 Force profiles of protein pulling with or without cytoskeletal links studied by AFM Biophys. Biochem. Res. Commun. 348 238–44

[27] Borghi N and Brochard-Wyart F 2007 Tether extrusion from red blood cell: integral proteins unbinding from cytoskeleton Biophys. J. 93 1369–79

[28] Bell G I 1978 Models for the specific adhesion of cells to cells Sci. 92 1541–55

[29] Evans E and Ritchie K 1997 Dynamic strength of molecular adhesion bonds Biophys. J. 72 1541–55

[30] Walton E B, Lee S and Van Vliet K J 2008 Extending Bell’s model: how force transducer stiffness alters measured unbinding forces and kinetics of molecular complexes Biophys. J. 94 2621–30

[31] Leckband D, Müller W, Schmitt F-J and Ringsdorf H 1995 Molecular mechanisms determining the strength of receptor-mediated intermembrane adhesion Biophys. J. 69 1162–9
[32] Sako Y, Nagafuchi A, Tsukita S, Takeichi M and Kusumi A 1998 Cytoplasmic regulation of the movement of e-cadherin on the free cell surface as studied by optical tweezers and single particle tracking: coralling and tethering by the membrane cytoskeleton J. Cell Biol. 140 1227–40

[33] Ward M D, Dembo M and Hammer D A 1995 Kinetics of cell detachment: effect of ligand density Annu. Biomed. Eng. 23 322–31

[34] Morris C E and Homann U 2001 Cell surface area regulation and membrane tension J. Membrane Biol. 179 79–102

[35] Evans E and Rawicz W 1990 Entropy-driven tension and bending elasticity in condensed-fluid membranes Phys. Rev. Lett. 64 2094–7

[36] Björnham O and Axner O 2009 Multipili attachment of bacteria with helix-like pili exposed to stress J. Chem. Phys. 130 235102