Cell blebbing and membrane area homeostasis in spreading and retracting cells

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Supporting Material for Cell blebbing and membrane area homeostasis in spreading and retracting cells

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I. MOVIE LEGENDS

Video S1: Fast bovine aortic endothelial cell (BAEC) detachment using 0.25% trypsin. Scale bar is 25µm.
Video S2: Slow detachment of BAEC using a 20:1 dilution of trypsin to BAEC medium. Scale bar is 25µm.
Video S3: BAEC blebbing cell plated immediately following trypsin detachment. Scale bar is 10µm.
Video S4: Non–blebbing BAEC that has incubated for 2 hours in solution prior to plating. Scale bar is 10µm.

II. MODEL

The theoretical model presented in the text gives a general description for the kinetics of cell adhesion that includes area recycling between the plasma membrane (PM) and area storage within membrane blebs (Eq. 1, main text). Cell spreading kinetics, quantified by the evolution of the cell area $A_c$ in contact with the substrate, is shown to be intimately related to the area $A_b$ sequestered in blebs at the cell surface, mostly controlled by the number of blebs $N_b$. All other variables introduced in the model, and in particular the various relevant tensions, are relative variables which are chosen to vanish in the reference state of a fully relaxed, non-adhering cell. Below, we give a detailed account of the mechanical model of bleb nucleation from which a relationship between the number of blebs $N_b$ and the membrane tension $\gamma_m$ can be derived, and give the explicit expression of the full system of dynamical equations describing the kinetics of cell spreading.

A. Bleb statistics

Blebs occur following a localized detachment of the PM from the cytoskeleton (CSK). This process is helped by the internal cell pressure, partly built by acto-myosin contraction, and is prevented by membrane-cortex adhesion and membrane tension. This can be formalized in a bleb energy $E_b$: $E_b = -PV_b + \gamma_m \Delta S_b + \epsilon_b S_b$, where $\epsilon_b$ is the binding energy between the PM to the CSK, $P$ is the pressure difference, and $V_b$, $S_b$ and $\Delta S_b$ are the volume in the bleb, the area of membrane-free CSK, and the area increase due to the presence of the bleb (Fig. 1). Although the following is qualitatively valid for blebs of any size, we concentrate for simplicity on low aspect ratio blebs $S_b/R_b^2 \ll 1$ where $R_b$ is the radius of curvature of the bleb, in which case this energy can be written:

$$E_b = \epsilon_b S_b + \frac{(\gamma_m - P R_b)S_b^2}{4\pi R_b^2}$$

(1)

Optimization with respect to the bleb curvature produces the Laplace law: $R_b = 2\gamma_m/P$, $E_b = \epsilon_b S_b - P^2 S_b^2/(16\pi\gamma_m)$. This energy shows a maximum for a given bleb area $S_b^*$, corresponding to a nucleation energy barrier $E_b^*$:

$$E_b^* = 4\pi\gamma_m\epsilon_b^2 / P^2, \quad S_b^* = 8\pi\gamma_m\epsilon_b / P^2$$

(2)

The smaller the barrier, the higher the probability to observe blebs on a given cell. Note that the nucleation energy is expected to be quite large (of order 1000k_BT for $\epsilon_b \sim 10^{-4} J/m^2$, $\gamma_m \sim 10^{-5} J/m^2$ and $P \sim 300$Pa), so spontaneous bleb nucleation is very unlikely to arise purely from thermal fluctuations. It has been suggested that a local increase of
pressure near the cell PM due to acto-myosin contractility provides the driving force for bleb nucleation \[2\]. Eq. \[2\] shows that the nucleation energy barrier can also be decreased by a drop of membrane tension. Since blebs on detached cells can persist for times consistent with the slow membrane spreading, we argue that they are a signature of a decrease of membrane tension.

The life cycle of a bleb involves nucleation and growth, followed by the polymerization of a new actin cortex underneath the bleb membrane and by bleb retraction. Following Kramer’s nucleation theory \[3\], the rate of bleb nucleation can be expected to depend exponentially on the energy barrier \(k_\text{n} = k_\text{n}^0 e^{-E_b/E_T}\) where \(k_\text{n}^0\) is the rate of bleb nucleation in the absence of energy barrier and \(E_T\) is the energy available from local fluctuations of the cell parameters (linkers concentration, local membrane tension, and local pressure). The rate of bleb retraction \(k_r\), on the other hand, depends mostly on the time needed to polymerize a cortical layer thick enough to retract the bleb, and can be assumed constant for simplicity. The simplest form of a kinetic equation for the evolution of the average number of bleb \(N_b\) per cell, that includes nucleation and retraction, reads:

\[
\frac{dn_b}{dt} = k_n(1 - n_b) - k_r n_b
\]

(3)

Where \(n_b \approx N_b S^*_0/A_{b0}\) is the fraction of cell area covered by blebs \((A_{b0}\) is the maximum total area in blebs), and where the average bleb area is assumed to be of order \(S^*_0\). A steady state is reached when bleb nucleation balances bleb retraction. From Eq. \(2\) one sees that the bleb nucleation energy increases linearly with membrane tension, so that the amount of cell blebbing is highly sensitive to membrane tension:

\[
n_b = \frac{1}{1 + e^{\beta(\gamma_m - \gamma_b)}} \quad \text{with} \quad \beta \equiv \frac{4\pi\rho_0^2}{E_T} \quad ; \quad \gamma_b = E_T \frac{P^2}{T_b} \log \frac{k_r}{k_n^0}
\]

(4)

where \(\beta\) is the bleb sensitivity to membrane tension and \(\gamma_0\) a threshold tension below which cell blebbing can be observed. Eq. \(4\) corresponds to Eq. 2 of the main paper, and is represented in Fig. 1, illustrating the fact that blebs are absent under high tension \(\gamma_m \gg \gamma_b\), and entirely cover the cell surface under low tension \(\gamma_m \ll \gamma_b\) (Fig. 1).

### B. Final equations

The viscoelastic model described by Eq. 1 (main text) and depicted in Fig. 4 (main text) includes an energy scale (per unit area: the adhesion energy \(\epsilon\)), two time scales (the spreading time \(\tau_c = \eta_c/k_2\), and the time for membrane area transfer \(\tau_m\)) and two stiffnesses \((k_1\) and \(k_2\), combining the stretching of the plasma membrane and of the membrane of internal organelles). To reduce the number of parameters, we express all tensions in unit \(\epsilon\), all times in unit of \(\tau_c\), and all areas in unit the adhered area at saturation: \(A_{c, sat}\) (controlled by the effective cell stiffness at long time \(k_2\): \(A_{c, sat} = \epsilon/k_2\)). The problem reduces to the set of normalized equations

\[
\dot{A}_c = \left(1 - \frac{n_b}{n_b^*}\right) - \gamma_m \quad ; \quad \dot{\gamma}_m + \frac{\gamma_m}{\tau_m} = \left(1 + \frac{k_1}{k_2}\right) \left(\dot{A}_c + \dot{A}_b\right) + \frac{\left(A_c + A_b - A_b(\gamma_m = 0)\right)}{\tau_m} \quad ; \quad A_b = \frac{A_{b0}}{1 + e^{\beta(\gamma_m - \gamma_0)}}
\]

(5)

where the adhesion energy has been modified to include the observed repulsive effect of blebs: \(\epsilon \rightarrow \epsilon(1 - n_b/n_b^*)\), \(n_b^*\) being a phenomenological parameter representing the bleb density for which the cell is unable to feel the adhesive nature of the substrate \((\epsilon = 0\) for \(n_b > n_b^*)\). The predictions of these equations in various situations of interest for the experiments presented in the text is discussed below.

### III. RESULTS

#### A. Area transfer and cell spreading

In the absence of blebs \((A_{b0} = 0)\) the model is linear and can be solved analytically. Within our framework, the spreading of a purely elastic cell follows an exponential saturation of the form \(A_c(t) \propto A_{\infty}(1 - e^{-1/\tau})\) and reach the saturation area \(A_{\infty}\) with a single timescale \(\tau\). With our normalization (Eq. \(5\), \(A_{\infty} = \tau = 1\) if membrane recycling is very fast \((\tau_m \rightarrow 0)\), and \(A_{\infty} = \tau_\infty = 1/(1 + k_1/k_2)\) if recycling is absent \((\tau_m \rightarrow \infty)\). Tension-controlled membrane recycling allows the cell to spread more, as shown in Fig. 4B (main text). The viscoelastic model that takes into account the finite time of area transfer between the PM and inner organelles exhibits a second timescale \(\tau_m\) characteristic of this transfer (Fig. 4B, main text). While the initial spreading kinetics is controlled by viscous dissipation in the cytosol (friction parameter \(\eta_c\)), the later stage is slower, controlled by the kinetics of area transfer (parameter \(\eta_i\)).

#### B. Blebs and membrane tension

Mechanically, blebs behave in a way reminiscent of smaller membrane outgrowth such as caveolae \(4\), which can regulate membrane tension by sequestering excess membrane area. This is illustrated during the rapid detachment of strongly adhered cells. The total PM area \(A_m\) (that includes blebs) increases by membrane transfer during cell spreading.
to match the increase of the apparent cell area $A_c$. Upon cell detachment, the cell quickly rounds up under the contractile cortical stress, without rapid change of the PM area. The subsequent increase of the PM excess area $A_m - A_c$ lowers the membrane tension, which might drop below the threshold $\gamma_b$, allowing for the formation of membrane blebs. By incorporating some of the excess area, blebs keep the membrane tension to a value close to $\gamma_b$. The tension can however drop further if the excess membrane area becomes so large that the cell surface is saturated by blebs ($n_b \simeq 1$).

While the formation of blebs upon cell detachment is not easy to monitor, one may quantify the disappearance of membrane blebs after cell detachment (on non-adhered cells) as membrane area transferred away from the PM slowly reduces the area excess (Fig. 5A, main text). With our experimental conditions, the number of blebs remains initially constant, which we interpret as the signature that immediately after rounding up, the detached cell is saturated with blebs and its tension is lower than the blebbing threshold. The subsequent decrease of the number of blebs follows the kinetics of membrane area transfer, until blebs completely disappear for the cell surface.

Quantitatively, the total PM area at spreading saturation can be obtained using $\gamma_m = \epsilon$ and $A_c = 0$ (in Eq. 1 - main text), to find $A_m, sat = \epsilon k_1 k_2 (k_1 + k_2)$. Very fast detachment occurs without change of PM area, and yields a strong reduction of membrane tension, to a value $\gamma_{m,detach}$ that decreases with increasing adhesion. One thus expects strong cell blebbing upon fast detachment, provided the following is satisfied:

$$\text{fast detachment : } \gamma_{m,detach} \simeq -\frac{k_1}{k_2} \epsilon \quad \text{Blebbing upon detachment if } \gamma_b > -\frac{k_1}{k_2} \epsilon$$

(6)

C. Spreading of a blebbing cell

Our experimental setup allows us to quantify the effect of blebs on cell spreading, since the number of blebs per cell can be controlled by changing the incubation time, as show in Fig. 5A (main text). We observe that blebs have a strong effect on the initial stage of cell spreading. Blebbing cells near a substrate only start spreading after a lag time that increases with the initial number of blebs. We propose that blebs act as pressurized cushions preventing strong cortex adhesion to the extracellular matrix. Part of this effect can be compared to the Helfrich repulsion of a thermally fluctuating membrane near a substrate [5], although the “fluctuations” of a blebbing cell are actively driven (by the acto-myosin cortex) and have a well-defined size $S_b$ (Eq. 2). It is in principle quite complicated to compute the effect of blebs on cell adhesion, since in addition to the “air bag” effect discussed above, some blebs near the substrate are seen to retract as they would in the absence of substrate, while others maintain a stable adhesion. Here, we use a very simple mean-field approach, and assume that the effective adhesion energy is reduced (linearly) when blebs are present: $\epsilon_{eff} = \epsilon (1 - n_b/n_b^*)$, where $n_b^*$ is the bleb occupancy that prevents adhesion. This fairly crude approximation manages to qualitatively reproduce the experimental observations, as we show below. We numerically solve Eq. 5 for different initial conditions corresponding to different incubation times (the white squares of Fig. 5A, main text), and obtain the spreading kinetics shown in Fig. 5B (main text). The duration of the lag phase observed in the experiment are reproduced by choosing $n_b^* = 1/4$, meaning that the cell is prevented from adhering to the substrate when blebs covered about 25% of its surface.