Liposome adhesion generates traction stress

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SUPPLEMENTARY TEXT AND FIGURES

Liposome Preparation

Liposomes are prepared by two methods: electroformation (EF) \(^1\) (Fig 1, 3, Movies 1,2, and 4) and inverted emulsion (IE) \(^2\) (Fig 2, 4, Supp Figs 1-7, Movies 3, 5-8). Substrate contraction and lipid membrane phase change occurs with both formation methods. With the inverted emulsion method however, membrane dynamics are slower\(^3\), allowing us to measure the shape of the liposome by confocal microscopy before it bursts (Within 15 minutes, approximately 71% IE liposomes rupture, versus 100% of EF liposomes). We describe the formation by both methods below.

The lipids used are a combination of neutral L-\(\alpha\)-phosphatidylcholine from egg yolk (EPC, 840051C), cholesterol (ovine wool, 110796), and 1,2-dioleoyl-sn-glycero-3-\{\[n(5-amino-1-carboxypentyl) iminodiacetic acid\]succinyl\}nickel salt (DOGS-NTA-Ni). All of these lipids were purchased from Avanti Polar Lipids (Alabaster, AL). For fluorescent contrast, we use Oregon Green or Texas Red 1,2-Dihexadecanoyl-sn-Glycero-3-Phosphoethanolamine (DHPE) from Molecular Probes (Invitrogen). The precise composition of the liposomes are mentioned in Table 1.

Buffers for the Electroformation of Liposomes. Internal Buffer: 100 mM sucrose. Outside Buffer: 100 mM glucose.

Buffers for Inverted Emulsion formation of Liposomes. Internal Buffer: 100 mM KCl, 4 mM CaCl\(_2\), 4 mM MgCl\(_2\), 10 mM HEPEs, 1 mM DTT, 0.5 mM DABCO, 10 mM ATP, 280 mM sucrose, 0.56 M dextran (40k MW), pH 7.0. The osmolality is adjusted to approximately 380 mmol/kg. External Buffer 1: 10 mM HEPEs (ph 7.5), 2mM MgCl\(_2\), 0.2 mM CaCl\(_2\), 1mM DTT, 0.5mM DABCO, 275 mM glucose, 0.01 mg/mL casein.
Adjusted pH to 7.0. Osmolality is adjusted to 380-400 mmol/kg. External Buffer 2: 10mM HEPES (ph 7.5), 2 mM MgCl₂, 0.2 mM CaCl₂, 1mM DTT, 0.5mM DABCO, and 275mM glucose and 0.6-1.2% methylcellulose (14,000 MW). Adjusted pH to 7.0. This buffer is adjusted to approximately 425 mmol/kg.

For electroformation (EF), lipids are combined as listed in Table 1 at a concentration of 2.5 mg/mL. 25 μl of this solution is then spread on two ITO conductive slides. Copper tape is applied to each slide, and the slides are sealed together using vacuum grease. The frequency is set to 10 Hz and the voltage is first set to 0.1 V, and increased to 0.2, and 0.3 V after 5 minutes each. Then voltage is then increased to 1V for 30 minutes. After 30 minutes, the frequency is dropped to 4 Hz, and is kept at 1V for another 30 minutes.

For the inverted emulsion (IE), phospholipids in the molar ratios described in Table 1 are mixed in a glass vial and dried under N₂ gas. Afterwards the chlorofom has evaporated, the lipids are dissolved in mineral oil (Sigma). The oil was then sonicated in a bath sonicator for between 10 and 30 minutes at room temperature. The oil was then heated to 50°C for 3 hours. The mixture is then cooled to room temperature and stored at 4°C for up to one week. Then 5μL of internal buffer is added to 250μL of mineral oil in a 0.5 mL eppendorf. This mixture is syringed 1-2 times. Separately, in a low absorption 0.5 mL eppendorf, 30 μL of mineral oil is added to the top of 70 μL of external buffer 1. Then, the emulsion is added to the top of the mineral oil layer in the low-absorption eppendorf. This mixture is then centrifuged at 100g for 15 minutes at 4°C. The osmotic pressure difference between the internal and external buffer 1 is between 0 and 20 mmol/kg. For the experiment, the 70 μL of external buffer 1
containing the liposomes is added to an imaging chamber containing 500 \( \mu \)L of External Buffer 2. The osmotic pressure difference between external buffer 1 and 2 is between 1 and 20 mmol/kg.

**Table 1: Composition of liposomes**

<table>
<thead>
<tr>
<th>Liposome (method)</th>
<th>EPC (%)</th>
<th>Cholesterol (%)</th>
<th>NTA (%)</th>
<th>DHPE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPC (IE)</td>
<td>53.8</td>
<td>36</td>
<td>10</td>
<td>0.2</td>
</tr>
<tr>
<td>EPC (EF)</td>
<td>99.6</td>
<td>0</td>
<td>0</td>
<td>0.4</td>
</tr>
</tbody>
</table>

**Adhesion**

The adhesion between the liposome and polyacrylamide gel is mainly mediated by the partial negative charges in the liposome due to the oxidized EPC lipids and the cationic poly-L-lysine (Sigma) bound to the gel. The poly-L-lysine is a long linear chain (MW>>70,000) generating a very high charge density on the gel surface. The strength of adhesion is modulated by varying the concentration of poly-L-lysine (0.1-10 mg/mL) and quantified by measuring the liposome/substrate contact angle by confocal microscopy. Small contact angles reflect a very adhesive ("wetting") surface, while large contact angles reflect a highly non-adhesive surface. Greater concentrations of poly-L-lysine on the gel correspond to smaller contact angles, thus mediating stronger adhesion between the liposome and substrate regardless of the stiffness of the substrate (Supp Fig 1).
To confirm that the deformations of the gel surface reflect those of the adherent liposome, we evaluate whether there is any slip between the substrate and the membrane. We quantify the motions of TR-DHPE within the liposome by PIV and observe that the magnitude of the radial displacement of the lipids corresponds exactly to that of the underlying substrate (Supp Fig 3). The substrate and membrane are thus well coupled at the adhesion surface.

**Polyacrylamide Gel Formulation**

Polyacrylamide gels are polymerized onto coverslip surfaces of 25mm diameter (#1.5, Electron Microscopy Sciences). Briefly, the coverslips are treated with a combination of aminopropyl silane and glutaraldehyde to make the surface reactive to the acrylamide. Varying concentrations of bis/acrylamide are mixed with 0.1mg/mL ammonium persulfate to yield a gel with an Elastic Modulus of 1.8 kPa to 165 kPa respectively ($\nu=0.5$, Table 2). 40 nm far red (647nm) beads are embedded in the gel mixture prior to polymerization. Before the gels are fully polymerized, 12 $\mu$l of the gel is added to the coverslip and covered with another coverslip which has been made hydrophilic through treatment with RainEx. After the gels are polymerized on the coverslips, they are reacted with the standard Sulfo-Sanpah protocol as previously published ⁴. The surface of the reactive gels is then coated with 10 mg/mL of poly-L-Lysine Hydrobromide of $\gg70k$ MW (MP Biomedicals) which has been resuspended in MilliQ, and the pH set to 9. The reaction proceeds for 1 hour in the dark, and the coverslips are then rinsed in 1X PBS.
Table 2. Bis/Acrylamide Gel Concentrations

<table>
<thead>
<tr>
<th>E (kPa)</th>
<th>% Acrylamide</th>
<th>% Bis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2</td>
<td>5</td>
<td>0.05</td>
</tr>
<tr>
<td>1.8</td>
<td>5</td>
<td>0.075</td>
</tr>
<tr>
<td>4.2</td>
<td>5</td>
<td>0.1</td>
</tr>
<tr>
<td>8.4</td>
<td>7.5</td>
<td>0.1</td>
</tr>
<tr>
<td>16.3</td>
<td>7.5</td>
<td>0.2</td>
</tr>
<tr>
<td>165</td>
<td>12</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Force Measurements

We compare the images of the gel-embedded beads before and immediately after the vesicle ruptures. The time leading up to the liposome rupture builds stress as compared to the position of the beads immediately after rupture where the beads have relaxed. We compare these two images to calculate a sub-diffraction-limited displacement of the beads due to traction by the lipid liposomes. The Displacement fields are calculated using Particle Image Velocimetry (PIV) that runs on Matlab (Mathworks) available at http://www.oceanwave.jp/software/mpiv/.

Microscopy

The 25mm gel-coated coverslips are sandwiched in a custom-built chamber from Chamlide Live Cell Imaging. Imaging of sample fluorescence is performed using a Ti-E microscope (Nikon) with a spinning disk confocal head (Yokagawa), an HQ2 Coolsnap CCD camera (Roper Scientific) and a 60x 1.4Na oil immersion objective lens (Nikon). The microscope is controlled using Metamorph software (MSD Analytical Technologies). The spreading of the liposomes was imaged by confocal microscopy acquiring at 488nm, the wavelength that excites the oregon green (OG) DHPE lipid or 568nm which excites the Texas-Red (TR) DHPE, and at 642 nm which corresponds to
the beads embedded in the acrylamide. All experiments were performed at 25°C unless otherwise noted.

**Image Analysis**

The spreading of the liposomes was quantified using custom-written matlab routines that measures the spread area over time from the confocal images (Mathworks). Liposome contact angles are measured by first displaying confocal z-stacks of adherent liposomes in a side-view as visualized using Imaris (Bitplane). Then, using the angle feature of ImageJ (NIH), the contact angles are measured by manually by drawing a triangle on the image and recording the contact angle. Deconvolution of three-dimensional z-stacks of both the lipid liposomes and the polyacrylamide gel is also done in ImageJ using the Deconvolve 3D plug-in.

**Calculation of the Real substrate-Liposome contact area**

The three-dimensional area of the liposome as bound to the substrate is given by

\[ A_{3D} = \pi \left( r^2 + u_z^2 \right) \]  

(Eqn 1)

where \( r \) is the contact radius of the liposome and \( u_z \) the maximal vertical deformation of the substrate. The two-dimensional, projected area reads \( A_{2D} = \pi r^2 \) and thus:

\[ A_{3D} = A_{2D} \left( 1 + \frac{u_z^2}{r^2} \right) \sim A_{2D} (1.00006) \]  

(Eqn 2)
Thus, the increase in area caused by the indentation (<0.01%) on a 1.8 kPa gel is much smaller than the decrease in area we see during adhesion (~3.5%), and the bilayer therefore undergoes a true compression.

**Detailed calculation of the deformation of the elastic substrate underneath the contact area**

We assume that the substrate is incompressible, so that its Poisson ratio is \( \nu = \frac{1}{2} \), and we calculate the elastic displacement \( \mathbf{u} \) of the substrate using the incompressibility condition \( \Delta \mathbf{u} = 0 \). We match the stress at the surface to the force exerted by the liposome on the substrate due to the excess pressure in the liposome and to the vertical component of the tension acting on the contact line. In the bulk of the substrate, the balance of elastic forces is written as

\[
\mu \nabla^2 \mathbf{u} - \nabla P = 0 \tag{Eqn 3}
\]

Where \( \mu = \frac{E}{2(1+\nu)} = \frac{E}{3} \) is the elastic shear modulus and \( P \) the pressure field insuring incompressibility. It is convenient to take the two dimensional Fourier transform with respect to the components \( (x,y) \) parallel to the surface of the substrate and to define the Fourier transform \( \tilde{\mathbf{u}} \ (\mathbf{q}, z) \). Combining the force balance (Eqn 1) to the incompressibility condition, the vertical component of the displacement satisfies:

\[
\frac{\partial^4 \tilde{u}_z}{\partial z^4} - 2q^2 \frac{\partial^2 \tilde{u}_z}{\partial z^2} + q^4 \tilde{u}_z = 0 \tag{Eqn 4}
\]

This equation must be solved with the following boundary conditions:

- deep in the substrate, the displacement \( \tilde{u}_z \) vanishes
on the substrate, the shear stress vanishes. This condition can be rewritten

\[
\frac{\partial^2 \tilde{u}^2}{\partial z^2} + q^2 \tilde{u} = 0.
\]

As explained in the main text, we only consider the limit where the deformation is dominated by substrate elasticity and we ignore surface tension. The normal stress \( \sigma_{zz} \) is then equal to the vertical force per unit area exerted by the liposome on the substrate. The Fourier transform of this force is:

\[
F(q) = -P_i \frac{2 \sigma_0}{q} \left( J_1(qr_0) - \frac{qr_0}{2} J_0(qr_0) \right)
\]  \hspace{1cm} (Eqn 5)

Where \( J_0 \) and \( J_1 \) are Bessel functions. The first term in the parenthesis is the force due to the excess pressure and the second term is the vertical force localized at the contact line. The total force exerted by the liposome vanishes so that \( F(q = 0) = 0 \).

The solution of Eqn 2 with these boundary conditions gives the deformation of the substrate surface:

\[
\tilde{u}_z(q, z = 0) = \frac{\pi r_0 P_i}{q \mu} \frac{J_2(qr_0)}{qr_0}
\]  \hspace{1cm} (Eqn 6)

where \( J_2 \) is the second order Bessel function. The deformation of the surface is obtained by inverse Fourier transformation. The value at the center of the liposome adhesion area where \( r = 0 \) is

\[
u_z(r = 0) = -\frac{P_i r_0}{4 \mu}.
\]
Supplemental Figure 1

**Liposome-Substrate Contact Angle is Greater on Stiff Substrates.** (a) Confocal z-stack of a liposome adherent to a polyacrylamide gel with Elastic Modulus (E) of 165 kPa. Estimation of the contact radius, \( r_0 \) versus the liposome radius, \( R_c \). (b) The “effective” contact angle, \( \theta_0 \), is measured identically for soft gels as for hard gels, from the apex of the gel at the contact line. The red square indicates the region zoomed-in and represented by a schematic on the right. (c) Contact angle of liposomes adhered to 165 kPa gels for varying concentrations of poly-L-lysine. (d) Effective contact angle adhered to the surface of poly-L-lysine coated polyacrylamide gels (E) 1.8 kPa and 165 kPa, formed at different initial osmotic pressures (dP_0) of 20 and 60 mmol/kg. Sample sizes are indicated on bar plot.
Supplemental Figure 2

Substrate is Indented After Liposome Adhesion. (a) Individual z-slices through the 1.8 kPa polyacrylamide gel beneath an adherent liposome moving in the positive z-direction. Fluorescence comes from the 40 nm embedded beads. (b) Height profile ($u_z$) as a function of radius from the center of the liposome, averaged over approximately 2.5 μm. (c) Boxplot of the distribution of $u_z$ ($r=0$) values for n=9 independent experiments. The red line indicates a mean value of 680nm.
No slip between Membrane and Substrate. (a) Overlay of two time points during liposome spreading on a 1.8 kPa polyacrylamide gel. Lipids are shown at 0s (beginning of stage P3) and 570s later, immediately prior to rupture. White arrows are overlaid, which show the displacement of the substrate embedded beads. (b) The displacement of the lipids is plotted against the displacement of the beads as measured by PIV.
Supplemental Figure 4

No Contraction Without Pressure Difference. (a) Brightfield images, (b) fluorescent images (TR-DHPE) in the membrane and (c) substrate-embedded beads during the adhesion of a ruptured liposome. The substrate stiffness is $E=1.8$ kPa. The red line in (a) indicates the contrast difference quantified in (d). Red arrow indicates the location of a pore. (d) Contact area over time for two liposomes where a pore has opened. (d, inset) Contrast change from the brightfield images in (a). (e) Traction Stress observed during the spreading of the liposome in (a-c).
Bud fusion occurs during liposome adhesion at 37°C. Quantification of the projected 2D area of buds dynamics during the adhesion of a liposome (EPC/NTA/Chol) on a 1.8 kPa gel (Movie 7). Each line represents the area of a separate bud, and the 'jumps' correspond to fusion events.
Supplemental Figure 6

**Adhesion Energy Increases During Spreading Due to Negative Lipid Accumulation.** After adhesion of liposomes to both soft (1.8 kPa, dotted line) as well as hard (165 kPa, solid line), the fluorescence intensity of Texas Red DHPE increases until it photo-bleaches and decays at long times. This implies there is excess lipid available to the contact area. Time $t = 0$ corresponds to the beginning of P3.
Supplemental Figure 7

**Liposome Adhesion Induces Lipid Phase Change on Soft Substrates.** Oregon Green-DHPE fluorescence in the contact zone of a liposome bound to a 1.8 kPa gel.
References


