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Dynamic Theory of Pearling Instability in Cylindrical Vesicles

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We give a simple theory for recent experiments of Bar-Ziv and Moses, in which tubular vesicles are excited using laser tweezers to a "peristaltic" state. Considering the hydrodynamics of a bilayer membrane under tension, we reproduce some of the qualitative behavior seen and find a value for the wavelength of the instability in terms of independently measured material parameters, in rough agreement with the experimental values.

10/94

Despite its vast complexity, the living world has always inspired physical scientists with its habit of choosing simple geometrical forms for its structures (see for example [1]). While the link between real living systems and simple models generating similar shapes is often tenuous at best, recent years have seen remarkable progress in explaining the shapes of structures such as normal and diseased red blood cells. While plasma membranes are mixtures of thousands of lipids and proteins, extremely simple artificial membranes consisting of a single lipid reproduce much of their shape behavior in accordance with equally simple mathematical models [2]. To date most work has focused on equilibrium shapes, but of course biological systems are usually not in equilibrium (*i.e.*, dead), and so both theoretical and experimental work has recently turned to dynamical shape problems.

Quasicylindrical shapes are also abundant in Nature, though they have been less studied than the bag-like shapes reminiscent of blood cells. For example, long ago Thompson remarked [1] a family resemblance between certain foraminifera and the shapes of constant mean curvature found by Delaunay [3]. These shapes are a "peristaltic" modulation of a cylinder, *i.e.* a periodic change in its diameter. More reasonably perhaps, Deuling and Helfrich called upon these shapes to explain the observed 'myelin figures' found inside and outside aged red blood cells [4]. Such modulated cylinders have also been seen in several recent experiments, for example refs. [5][6]; a more extreme form consisting of large pearls on a string exists as well [5][7].

We will focus on the beautiful work of Bar-Ziv and Moses, who excited long cylindrical lipid bilayer vesicles using laser tweezers [5]. Their experiment seems unique in the degree of control over the circumstances of excitation; time scales can readily be measured and the role of thermal fluctuations is clearly seen via video microscopy. Briefly the observed phenomenon of interest to us is as follows. Initial preparation of the system yields stable tubular structures with a wide variety of radii R_0 between 0.3–5μm. The tubes are nearly straight cylinders some hundreds of microns long, anchored at both ends by large globules of lipid. Each tube consists of a single bilayer of DMPC or DGDG. The high temperature used ($\sim 45°$C) precludes any in-plane ordering of lipid molecules, so that away from the localized excitation a pure fluid membrane model suffices to describe their shapes. Initially the system is somewhat flaccid, as seen from visible thermal undulations and the fact that the tubes are not quite straight.

Application of a laser spot localized to $\sim 0.3\mu$m produces a dramatic transformation to the peristaltic shape. Greater laser power is required for larger tubules. Once formed, the peristaltic shape has a well-defined wavelength λ_0 which is uniform over dozens of wavelengths. Whatever the initial radius R_0, λ_0 is found to be $2\pi R_0/k_0$ where the dimensionless wavenumber k_0 is always between 0.64 and 1, and typically about 0.8. After prolonged tweezing some buildup of lipid becomes visible at the point of application of the laser. As the modulation grows more pronounced, k grows from k_0 to become slightly greater than 1. The modulated state is *tense*: visible thermal fluctuations are suppressed and the tube draws itself straighter than initially.

In this Letter we give a dynamical theory for the initial pearling instability. In particular we will show how to compute the preferred wavenumber k_0 in terms of R_0 and independently measured material parameters. Our model is an elaboration of the suggestion in [5] that the instability is of Rayleigh type, but with some significant changes to Rayleigh's classic analysis [8]. For example, the Rayleigh mechanism predicts $k_0 = 0$. Our mechanism boils down to a competition between a driving force, membrane tension induced by the laser, and ordinary hydrodynamic drag. After a simple back-of-the-envelope calculation we will add to our picture the bending and stretching moduli for the bilayer membrane as well as the friction between its two leaves. On short time scales the friction tends to lock the layers together [9][10], leading to a transient effective spontaneous curvature. Using independently measured values for all the material parameters we will then get k_0 in rough agreement with experiment. More details will appear elsewhere [11].

We cannot simply identify the modulated cylinders as equilibrium surfaces of constant mean curvature, since nothing in the problem selects a curvature. Unlike ref. [4] we have no *chemical* asymmetry between the inner and outer fluids to generate a tendancy to bend, and hence no spontaneous curvature. Indeed the tubules are polydisperse, each one's radius being set by the distance L_0 between terminal globules and the volume which happened to get trapped during formation. Nor do we have a fixed *pressure* difference as imagined in [1], which could have set a curvature by a Laplace-type law. Nor can we appeal to an area-difference *strain* frozen in at formation (unlike closed vesicles [12][13][14]); each layer of the tubule is initially in equilibrium with a common reservoir, the terminal globule, and this implies that the effective spontaneous curvature vanishes [12].[1] Finally, the Delaunay shapes [3][1] have an initial instability at wavelength $k = 1$ [11], larger than any observed value. In fact we cannot understand the observed shapes as equilibrium shapes for some suddenly modified elastic energy, so we turn to dynamics.

[1] Actually this is advantageous, as our results do not depend on an unobservable parameter varying from one tubule to the next.

The most famous dynamic instability in cylindrical geometry is that of a column or jet of water in air [15]. Despite a superficial resemblance to the pearling instability, however, Rayleigh's original analysis does not apply to the micron scale, where water is viscid. Following Plateau [16], Rayleigh later showed that for a cylinder of viscid fluid (a 'thread of treacle') in air, interfacial tension gives a fastest-growing unstable mode at $k_{\rm max} = 0$ [8], which is far from the observed k_0. In fact Rayleigh's answer depends on a boundary condition which was appropriate for his problem but not for ours; imposing no-slip boundary conditions at a nearly incompressible membrane instead of Rayleigh's condition of no tangential force will give us $k_{\rm max} \neq 0$ (see also [17]). We will then justify this simple model by incorporating the full elastic and dynamic structure of the membrane.

To get started let us consider the nature of the excitation. Initially our membrane is under almost zero tension, as seen from the thermal motion. When the laser comes close to the membrane, nothing happens: local heating is not important. When the laser spot *touches* the membrane, it pulls material in by the usual tweezer effect. While it is hard to calculate the exact tension so induced, we may easily estimate it as follows[2]: the applied laser power of ~ 50mW, spread over a spot of diameter 0.3μm, corresponds to an energy density in vacuum $\mathcal{E}$ of $3 \cdot 10^4 \mathrm{erg\,cm}^{-3}$. Taking the dielectric contrast between water and lipid at this frequency to be of order $\delta\epsilon = 0.23$ [18], we see that when a lipid molecule falls into the trap displacing water we gain an energy [19] $\sim \mathcal{E}\delta\epsilon \cdot a_0 D$, where a_0 is the area of the molecule's head and D is its total length. Taking $2D \sim 40$Å, we get that each unit of bilayer area sucked into the trap yields an energy gain of $\Sigma \sim 10^{-3} \mathrm{erg\,cm}^{-2}$. While this figure is probably an overestimate, we see that the trap generates a tension well in excess of the critical value [5] $\Sigma_{\rm crit} \sim \frac{\kappa}{R_0^2} \sim 10^{-4} \mathrm{erg\,cm}^{-2}$ needed to trigger shape transformations, where $\kappa \sim 0.6 \cdot 10^{-12}$erg is the bending stiffness of DMPC bilayers and we took $R_0 = 0.7\mu$m for illustration. Let $\sigma \equiv \Sigma R_0^2/\kappa$ denote the dimensionless tension; thus $\sigma \sim 10$. Following [5] we will take this tension to be distributed uniformly over the whole surface, even though in fact it propagates outwards from the laser spot. If the laser is removed the tension reverts back to zero, thermal fluctuations resume, and the tubule relaxes back to its initially stable cylindrical shape, as observed.

We work in cylindrical coordinates and describe our shape by the locus $r = R_0(1 + u(z,t))$ where $|u| \ll 1$. (Non-axisymmetric perturbations turn out to be stable [11].) For

[2] We thank R. Bruinsma for suggesting this estimate. Any heating caused by direct contact with the membrane will simply increase slightly the area in the trap without changing the desire of molecules outside the trap to get in.

our linearized analysis we can treat each Fourier mode separately, except the constant term u_0. Since u_0 is the only mode which can decrease the volume, volume conservation in an infinite cylinder requires (see [11]) that we choose $u(z) = -(u_k)^2 + 2u_k \cos(kz/R_0)$, where we truncate all formulas to $\mathcal{O}(u^2)$ and u_k is a function of time which we are to find. Using the area element [20] $\mathrm{d}S = [1 + u + \frac{1}{2}R_0^2(\nabla u)^2]\, R_0\, \mathrm{d}z\, \mathrm{d}\phi$, we at once see that this perturbation decreases the area $A = \int \mathrm{d}S = A_0[1 + (k^2 - 1)(u_k)^2]$ only for $k < 1$ [16]. Hence membrane tension cannot destabilize modes with $k > 1$, as observed. For $k < 1$, the laser does work $-\delta A \cdot \Sigma$ on our system as the modulation u_k grows.

Where does this energy go? On micron scales we may ignore kinetic energy, but some energy will go to viscous dissipation inside and outside the tube. As mentioned, energy can also go into the internal structure of the bilayer, for example the bending elasticity, but let us neglect such complications for our first estimate. As our vesicle changes shape, conservation requires a central flow velocity $v_z(r = 0) \sim (R_0/k)\dot{u}_k$ to transport the fluid from the troughs to the crests. But membrane incompressibility and no-slip boundary conditions between the layers and adjacent fluid require a much smaller value of $v_z(r = R_0) \sim 0$ at the boundary. Thus we get a velocity gradient and a shear dissipation of $2A_0R_0\eta\Lambda(k)^{-1}\dot{u}_k^2$, where η is the viscosity of water and the dynamical factor $\Lambda(k)$ is proportional to k^2 at small k. Equating this power loss to the gain $-\dot{A}\Sigma$ yields a growth rate

$$\gamma_k \equiv \dot{u}_k/u_k = \Sigma\Lambda(k)(1 - k^2)/R_0\eta \quad . \tag{1}$$

Approximating $\Lambda(k)$ by its small-k (Poiseuille) form, we see that γ reaches its maximum at $k_{\mathrm{max}} = 1/\sqrt{2}$, right in the experimentally observed range. A more exact solution of viscid hydrodynamics inside and outside a moving boundary with incompressible-layer boundary conditions gives [11]

$$\Lambda(k) = -\frac{1}{2k}\frac{[k(K_0^2 - K_1^2) + 2K_0K_1][k(I_0^2 - I_1^2) - 2I_0I_1]}{2I_0K_0/k + k(I_1^2K_0^2 - I_0^2K_1^2)} \quad , \tag{2}$$

where I_ν, K_ν are the usual Bessel functions [19], evaluated at k. With this Λ we get $k_{\mathrm{max}} = 0.68$. Note that k_{max} is a purely geometric constant because we cannot form any length scale from the tension and viscosity. We also see from (1) that the tension Σ, and hence laser power, needed to get noticeable growth rate increases with tubule radius R_0, as observed [5].

We now outline how to account for the internal dynamics of the membrane, and when these will be important to our problem. Symmetric bilayers resist bending and stretching with an elastic energy which we may write as [10][11]

$$F[H,\phi^+,\phi^-] = \int \mathrm{d}S \frac{\kappa}{2}\left\{(2H)^2 + \frac{\beta}{2d^2}\Big[\big(\frac{\phi^+}{\phi_0}-1\big)^2 + \big(\frac{\phi^-}{\phi_0}-1\big)^2\Big]\right\} \quad . \tag{3}$$

Here H is the mean curvature of the bilayer midplane and $\phi^\pm$ are the lipid densities of outer (respectively inner) monolayers, measured at the neutral surfaces of each monolayer. κ is the usual static bilayer bending stiffness, $d \sim D/2$ is the distance from the bilayer midplane to the monolayer neutral surface [21], and $\beta \equiv Kd^2/\kappa$, where K is the bilayer compression modulus. For DMPC, $\beta \sim 3.5$ [14]. We have dropped the topological term as usual. In equilibrium $\phi^\pm$ can adjust to their preferred values and we recover the usual curvature model. More generally, let us rephrase (3) in terms of the densities $\psi^\pm = \phi^\pm(1 \mp 2Hd)$ referred to the bilayer midplane. Initially we have $\psi^\pm = \psi_0^\pm \equiv \phi_0(1 \mp 2H_0 d)$, but after excitation $\psi^\pm$ can change along with H. Letting $\rho^\pm$ be the relative density change, $\rho^\pm = \psi^\pm/\psi_0^\pm - 1$, the density term of (3) becomes $\frac{K}{4}\big[(\rho^+ + 2(\delta H)d)^2 + (\rho^- - 2(\delta H)d)^2\big]$ where $\delta H = H - H_0$ is the change of curvature.

Besides hydrodynamic drag and elasticity, there is one more sink of energy, the inter-layer friction. To estimate the importance of this resistance, write the frictional force per unit area as $b(\tilde v_+ - \tilde v_-)$ where b is a constant and $\tilde v_\pm$ are the tangential layer velocities [9][22][10]. Then

$$R_1 \equiv bd^2/\eta \tag{4}$$

is a new crossover length scale in the problem [10]. For length scales much smaller than R_1, bilayer friction can dominate hydrodynamic dissipation unless the former somehow vanishes. In this high-friction regime the hydrocarbon chains temporarily lock together, so that under a sudden disturbance the membrane acts like a thin plate, remembering its initial curvature.[3] Both diffusion measurements [23] and tether-pulling experiments

[3] The reader may wonder how to reconcile this effect with the appearance of very thin tethers in the extreme nonlinear regime [5]. Note that while these tethers have greatly increased curvature, still their *area* is so small that frictional losses incurred in their formation are not too great. We also note in passing that the radius of the long tethers in ref. [9] is related to the tension by $\Sigma = \kappa/R^2_{\mathrm{tether}}$. Substituting the tension used here into this relationship gives a tether radius roughly comparable to that seen in [5].

[9] yield $bd^2 \sim 10^{-6}\mathrm{erg\,sec\,cm^{-2}}$ while the viscosity $\eta \sim 10^{-2}\mathrm{erg\,sec\,cm^{-3}}$, so we get $R_1 \sim 1\mu\mathrm{m}$, comparable to our system's size R_0.

The projected density fluctuations $\rho^\pm$ are related to $\tilde{v}_\pm$ by conservation[4], $\nabla_\parallel \cdot \tilde{v}_\pm = -\dot{\rho}^\pm$. While friction thus obstructs changes in $\rho^+ - \rho^-$, nothing prevents $\rho^+ + \rho^-$ from quickly adopting its preferred value of zero and we finally obtain the quadratic approximation to the energy

$$F[H,\hat{\rho}] = \frac{\kappa}{2R_0^2}\int \mathrm{d}S\left[\mathcal{P}(k^2)u^2 + \tfrac{\beta}{4}\hat{\rho}^2 + \beta(1-k^2)\hat{\rho}u\right] \quad , \tag{5}$$

where $\hat{\rho} \equiv (R_0/d)(\rho^+ - \rho^-)$ and

$$\mathcal{P}(k^2) \equiv \tfrac{3}{2} - \sigma + (\sigma - \tfrac{1}{2})k^2 + k^4 + \beta(1-k^2)^2 \quad . \tag{6}$$

Normal force balance now sets $T^+_{rr} - T^-_{rr} = R_0^{-1}\cdot\delta F/\delta u$, where $T^\pm_{ij}$ are the fluid stress tensors outside (respectively inside) the vesicle, evaluated at the boundary. In linear approximation we have $T^+_{rr} - T^-_{rr} = -\eta\Lambda(k)^{-1}\dot{u}$, where $\Lambda(k)$ is the factor quoted in eqn. (2).[5]

To solve for both $u(z,t)$ and $\hat{\rho}(z,t)$ we must supplement the normal force-balance equations by the difference between the two tangent force-balance equations [10]: $-b(\tilde{v}^\pm - \tilde{v}^\mp) = \nabla_\parallel(\delta F/\delta\rho^\pm)$. We have retained only interlayer friction on the left side, since it turns out to dominate both the 2d viscosity [25][10][11] and the traction $\pm T^\pm_{rz}$ of the fluid [10][11]. Subtracting the two equations and using the lipid conservation equations gives us $\partial\hat{\rho}/\partial t = -(k^2/bd^2)\,\delta F/\delta\hat{\rho}$, where again $\hat{\rho} \equiv (R_0/d)(\rho^+ - \rho^-)$. We combine the force balance equations using (5) to get

$$\frac{\partial}{\partial t}\begin{pmatrix} u \\ \hat{\rho}\end{pmatrix} = -\frac{\kappa}{R_0^3\eta}\begin{pmatrix}\Lambda(k) & \\ & k^2R_0/R_1\end{pmatrix}\begin{pmatrix}\mathcal{P}(k^2) & \frac{1}{2}\beta(1-k^2) \\ \frac{1}{2}\beta(1-k^2) & \beta/4\end{pmatrix}\begin{pmatrix} u \\ \hat{\rho}\end{pmatrix} \quad . \tag{7}$$

The desired growth rate is the positive eigenvalue, if any, of this linear problem, *i.e.* $\gamma \approx (-180\,\mathrm{sec}^{-1})\cdot\lambda$, where we took a typical $R_0 = 0.7\mu\mathrm{m}$ and λ solves $\lambda^2 - \lambda(g_1+y) + g_0 y = 0$. Here $g_0 = \Lambda(k)[\frac{3}{2} - \sigma + k^2(\sigma - \frac{1}{2}) + k^4]$ is the growth rate with zero friction and $g_1 = g_0 + \Lambda(k)\beta(1-k^2)^2$ is the growth rate at infinite friction; $y \equiv \beta R_0 k^2/4R_1$.

[4] In general, conservation laws on moving curved surfaces are tricky (see [24]), but for our linearized analysis this treatment suffices.

[5] We may neglect the small boundary velocities $\tilde{v}^\pm \sim \dot{u}d \ll \dot{u}R_0$ in this part of the calculation [11].

Using measured values of the parameters quoted above, (7) with (2) yields a broad distribution of growth rates peaked around $k_{\rm max} \approx 0.65$ for any tension σ near our estimate $\sigma \sim 10$, which is consistent with some of the observations and certainly better than the $k_{\rm max} \sim 0$ found in Rayleigh's problem [8]. (Alternately the value of σ can be inferred from the observed growth rate $\gamma_{\rm max}$, once this is measured.) We also see why the naive treatment we gave first is so accurate: for large tension we have $g_0 \approx g_1$ and the growing eigenvalue $\lambda \approx \sigma(k^2-1)\Lambda(k)$ becomes completely insensitive to the friction. Physically, at large tension both the bending stiffness and the transient spontaneous curvature due to the layer friction become unimportant. To obtain the shorter wavelengths seen in other observations we must either suppose that in those cases the tubule had already passed into its nonlinear regime or that the effective values of the friction coefficient b and the area-difference elasticity parameter β are somehow larger than our estimates. Of course there may be still more physics which we have missed. For example we have not attempted here to study the propagation of the disturbance from a point source.

In short, direct application of laser tweezers can serve as a tool for exploring the dynamics of membranes by triggering visible shape transformations, complementary to other controlled techniques such as tether-pulling. We have seen how the laser-membrane interaction may be modeled by a very simple mechanism. We found that the membrane acts as a nearly incompressible boundary for the surrounding solvent, which significantly changes the instability of cylindrical geometry from the Rayleigh case in ways which have been observed. Analysis similar to that presented here should also prove useful in understanding other dynamical problems involving shape changes of vesicles, and in particular problems at lower tension, where the internal membrane dynamics will play a larger role. One may hope that the insight thus gleaned can find uses in understanding processes, such as vesiculation, of more direct biological interest.

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References

[1] D. Thompson, *On growth and form* (Cambridge, 1917).

[2] R. Lipowsky, "The conformation of membranes," Nature **349** (1991) 475.

[3] H. Bouasse, *Cours de mathématiques générales* (Paris, Delagrave, 1911).

[4] H. Deuling and W. Helfrich, "A theoretical explanation for the myelin shapes of red blood cells," Blood Cells **3** (1977) 713.

[5] R. Bar-Ziv and E. Moses, "Instability and 'pearling' states produced in tubular membranes by competition of curvature and tension," Phys. Rev. Lett., **73** (1994) 1392.

[6] S. Chiruvolu, *et al.*, "A new phase of liposomes: entangled tubular vesicles," Science, in press (1994).

[7] B. Thomas, private communication.

[8] Lord Rayleigh, "On the instability of a cylinder of viscous liquid under capillary force," Phil. Mag. **34** (1892) 145.

[9] E. Evans, A, Yeung, R. Waugh, and J. Song, "Dynamic coupling and nonlocal curvature elasticity in bilayer membranes," in *Structure and conformation of amphiphilic membranes,* ed. R. Lipowsky *et al* (Springer, 1992).

[10] U. Seifert and S. Langer, "Viscous modes of bilayer membranes," Europh. Lett. **23**(1993)71; "Hydrodynamics of membranes: the bilayer aspect and adhesion," Biophys. Chem. **49** (1994) 13.

[11] P. Nelson, T. Powers, and U. Seifert, to appear.

[12] E. Evans, Biophys. J. **14** (1974) 923; W. Helfrich, "Blocked lipid exchange in bilayers and its possible influence on the shape of vesicles," Z. Naturforsch. **29C** (1974) 510.

[13] U. Seifert, K. Berndl, and R. Lipowsky, "Shape transformation of vesicles," Phys. Rev. **A44** (1991) 1182; L. Miao *et al.*, "Equilibrium budding and vesiculation in the curvature model of fluid membranes," Phys. Rev. **A43** (1991) 6843

[14] L. Miao *et al.*, "Budding transitions of fluid-bilayer vesicles," Phys. Rev. **E49** (1994) 5389.

[15] Lord Rayleigh, "On the instability of jets," Proc. Lond. Math. Soc. **10** (1879) 4.

[16] J. Plateau, *Statique experimentale et theorique des liquides soumis aux seules forces moleculaires* (Gautier-Villars, 1873).

[17] R. Bruinsma, "Growth instabilities of vesicles," J. Phys. II France **1** (1991) 995.

[18] J. Israelachvili, *Intermolecular and surface forces* (Academic Press, 1992).

[19] J. D. Jackson, *Classical Electrodynamics* (Wiley, 1975) pp. 107, 160.

[20] Ou-Yang Zhong-can and W. Helfrich, "Bending energy of vesicle membranes," Phys. Rev. **A39** (1989) 5280.

[21] R. Waugh, J. Song, S. Svetina, and B. Zeks, "Local and nonlocal curvature elasticity in bilayer membranes by tether formation," Biophys. J. **61** (1992) 974.

[22] E. Evans and A. Yeung, "Hidden dynamics in rapid changes of bilayer shape," Chem. Phys. Lipids, in press.

[23] R. Merkel, E. Sackmann, and E. Evans, J. Phys. (Paris) **50** (1989) 1535.

[24] W. Cai and T.C. Lubensky, "Covariant hydrodynamics of fluid membranes," Phys. Rev. Lett. **73** (1994) 1186.

[25] E. Evans and R. Skalak, *Mechanics and thermodynamics of biomembranes* (CRC Press, 1980).