Mechanical characterization of elastic membranes: Cell mechanics applications

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Wrinkled patterns produced by cells crawling onto elastic membranes provide a useful tool to test living cells' locomotion. The technique, however, requires a characterization of the intrinsic properties and tension of the membrane as well as an accurate determination of the deformation induced by the cell. Here, the authors present a capillary-type technique to estimate the elastic modulus of such membranes, describe an indentation technique to measure the initial tension, and develop a simple interferometric method to measure the amplitude field of the wrinkled pattern produced, for instance, by the action of living cell. © *2007 American Institute of Physics.*
[DOI: 10.1063/1.2437672]

Wrinkling in elastic films under stress is ubiquitous in nature.[1–3] Common systems exhibiting this phenomenon are our skin,[4] textiles, and more specifically thin coatings subjected to stress mismatch.[5,6] Apart from a basic interest, wrinkled membranes have found unexpected applications recently. For instance, in modern technology, prewrinkled conductive films are proposed as stretchable electrical contacts required in large-area electronics.[7] In medicine, surgery has always involved cutting, thickening contraction, and in many cases wrinkling of the skin. Potential applications of wrinkle mechanics to improve wound healing have been recently summarized in Ref. 4. In turn, in cell biomechanics, wrinkled patterns produced by cells crawling onto elastic membranes have provided a useful tool to test living cells' locomotion. With this method, in which membranes are obtained by vulcanization of the free surface of polymeric fluids, the wrinkle length has been correlated to the force applied by the cell cytoskeleton.[1,8] In a recent article,[9] we reported an experimental and theoretical study of wrinkles formed in an initially flat elastic membrane, locally subjected to an axisymmetric tangential force. We show that wrinkles appear as a consequence of a supercritical instability: both the length and amplitude of the wrinkles increase as the imposed force is increased beyond a well defined critical value which is a function of the membrane tension. Beyond the instability threshold, the number of nucleated wrinkles is nearly independent of the applied force, varies very slowly with membrane tension, and is a linear function of the size of the object responsible for the wrinkling, i.e., the typical dimension of the region (relative to the membrane thickness) in which the cell attaches to the substrate. By contrast, the wrinkle length and amplitude proved to be very sensitive to the membrane tension, both quantities decreasing as the tension is increased. Moreover, the length resulted a slowly varying function of applied force, which challenges the sensitivity of cellular force measurements based on the direct measurement of the length. We suggested thus that a consid-

erable more sensitive measurement can be obtained if the force is correlated directly to the amplitude instead. However, such a program requires the development of simple techniques to carry out film characterization and wrinkle topography measurements. To overcome these difficulties, here we present a protocol to characterize elastic membranes used in cell locomotion measurements and we develop an interferometric method to account for the wrinkle amplitude.

The membranes are prepared by vulcanization, as described in Refs. 1 and 8, of a polymeric fluid, either phenyl-methyl polysiloxane (PMP, Dow Corning 710) or polydimethyl siloxane (PDMS, Sylgard 184). First, a thin fluid film is formed by spin coating on a clean piece of silicium wafer (Si: B, [111], P/E): A thickness of about 20 μm is achieved after spinning at 4500 rpm for 2 min. Then, an elastic thin membrane, a few nanometers in thickness, is produced at the free surface by the direct effect of a homogeneous flame from a bunzer-type burner. In order to ensure the final membrane homogeneity, the silicium sample is displaced at constant speed by means of a motorized mount, the contact time of the flame with the fluid surface being of about 1 s.

The first relevant quantity necessary for the interpretation of the wrinkled pattern is the membrane thickness, h. However, in our experimental configuration, nondestructive measurements are difficult. For instance, optical methods do not provide enough contrast as the membrane floats on top of a fluid having similar refraction index. Hence, we propose to make use of an atomic force microscope. However, such a technique necessitates to separate the membrane from the liquid, which we propose to achieve by transferring, by mechanical contact, onto a clean piece of silicium wafer (Fig. 1). The fluid, which may remain at the new created surface, is removed by spinning the whole. The cleaning process is improved by adding a small drop of an appropriate solvent (isopropilic ethanol). Immediately after spinning in order to avoid contamination, we measure the difference in height between two regions across the film edge by means of an atomic force microscope (AFM) in the contact mode (Fig. 1). The preparation protocol described above is found to lead
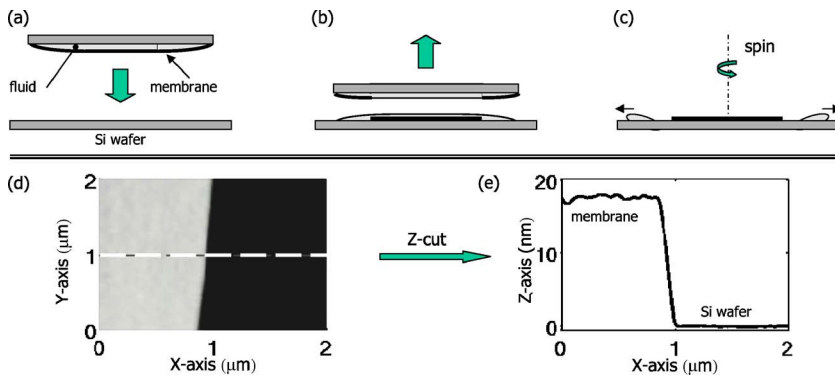
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FIG. 1. In order to obtain a clean membrane deposited on a silicon wafer, we approach the free surface of the sample from the solid surface (a), which results in a piece of membrane underneath a thin fluid layer (b). The remaining fluid is removed by spinning at large velocity (c). The thickness h [here (17.8 ± 0.2) nm] is measured at one edge of the membrane [image in the optical microscope (d)] which vertical profile (e) is obtained by using the AFM in the contact mode.

to PMP (PDMS) membranes having reproducible thicknesses $h=(18\pm1)$ nm $[h=(60\pm2)$ nm$]$.

The second relevant quantity is the two dimensional elastic modulus $B\equiv Eh/(1-\sigma^2)$ associated with the stretching of the membrane (we denote σ the Poisson ratio and E the Young modulus): In principle, provided that the membrane is thick enough, B can be determined from the force-displacement relation or Hertz law,[10] $F(\delta)=\frac{4}{3}[E/(1-\sigma^2)]\sqrt{r}\delta^{3/2}=\frac{4}{3}B(\sqrt{r}/h)\delta^{3/2}$, observed during the indentation of the dry membrane lying on the solid substrate with the AFM tip [Fig. 1(c), r stands for the radius of curvature of the AFM tip]. However, the relation holds true only when the indenter is pushed against an elastic half-space: In our experimental configuration, the membranes are so thin that the measurements are inaccurate and altered by finite size effects (the thickness h compares to r). We propose thus the capillarity-type method sketched in Fig. 2 instead. By using the vulcanization protocol described above, an initially flat membrane is prepared at the open end of a tube (radius $r_0=1$ mm) filled with the polymeric fluid [Fig. 2(a)]. We then impose a pressure difference, ΔP, across the membrane and, imaging the system from the side, we measure the resulting curvature $\mathcal{C}$ of the free surface. In the case of a liquid interface, the Laplace law would apply and

$\Delta P=2\Gamma\mathcal{C}$, with $\mathcal{C}$ the curvature of the interface cross section and Γ the surface tension (or free energy). In the case of an elastic membrane, the tension of the interface is not a thermodynamical quantity, which we shall denote T_0, in order to avoid confusion. For a membrane, the minimization of the elastic energy leads to a nonlinear relation between the pressure difference ΔP and the curvature $\mathcal{C}$,

$$\Delta P \approx 2T_0\mathcal{C} + 2.66B\frac{\arcsin(\mathcal{C}R) - \mathcal{C}R}{R}, \qquad (1)$$

where the elastic term, proportional to B, accounts for the increase in the tension resulting from the increase in the surface. Thus, the measurement of the nonlinear term provides us with an estimate of the elastic modulus B [Fig. 2(b)]. We get $B=(240\pm10)$ mN/m $[T_0=(60.5\pm0.5)$ mN/m$]$ for the PDMS membrane, and $B=(20\pm10)$ mN/m $[T_0=(27\pm1)$ mN/m$]$ for the PMP membrane. We mention that we can estimate from the values of B the Young modulus $E\simeq3$ MPa $(E\simeq0.8$ MPa$)$ of the PDMS (PMP) membranes.

The third relevant quantity is the tension T_0 of the membrane. As T_0 depends on the preparation of the system, the measurements from the capillary-type method are not applicable for the samples used in the biology experiment, and we propose here *in situ* measurements, instead. We choose to push vertically on the flat membrane with the suitable tip [Fig. 2(c)]. In this case, the force-displacement relation $F(\delta)$ satisfies,

$$F \approx \xi B^{1-\nu}T_0^{\nu}\delta, \qquad (2)$$

where $\xi=1.143\pm0.002$ and $\nu=0.881\pm0.003$ have been obtained by numerically solving the elasticity problem. As an example, we made use of a microneedle to measure the force-displacement relation in the same experimental condition as in Fig. 2(c) for the PDMS membrane. The slope indicates $T_0\simeq64$ mN/m, in fairly good agreement with the value $T_0\simeq60.5$ mN/m obtained from the pressure-curvature relation [Fig. 2(b)].

Finally, in the biology experiment, the geometry of the wrinkle pattern can be characterized by using a Michelson-type interferometer in which the sample acts as one of the mirrors. We made use of two objective lenses (Carl Zeiss CP Acromat, $10\times/0.25$), a dichroic cube of 25 mm side, a discharge Na source, and a video camera (Nikon DXM 1200, 12 Mpixel). The sample consists of fibroblast-type cells, cultured following the standard procedure described in Ref. 8, attached to a PMP membrane prepared as described above on a silicon wafer. An O ring around the substrate and a cover glass form a narrow container, about 0.5 mm depth, for the
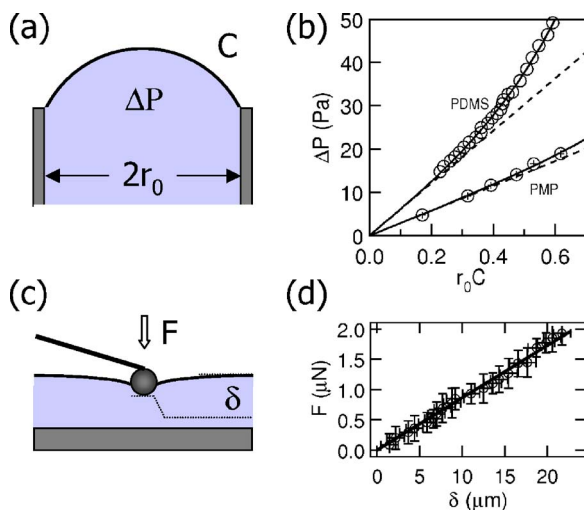

FIG. 2. Pressure difference ΔP is imposed, by using the principle of communicating vessels, across an initially flat membrane prepared at the upper end of a capillary tube. (b) Elastic modulus B associated with the stretching of the membrane is obtained from the nonlinearity in the relation between ΔP and the resulting curvature $\mathcal{C}$. (c) In the case of a membrane floating on a flat liquid layer, one can obtain in situ measurement of the tension T_0 by pushing vertically the free surface by means of a rounded tip. (d) Force F is proportional to the displacement δ, which is accounted by Eq. (2).
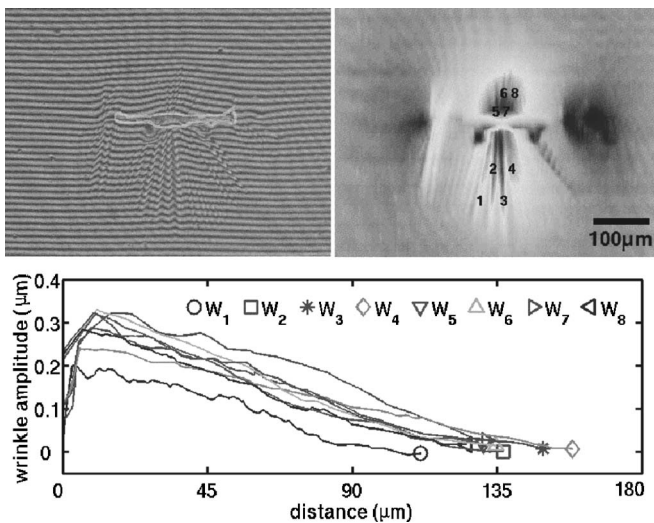
FIG. 3. Wrinkle pattern results from the contraction of a single fibroblast which is moving freely on the elastic surface. Left panel: equal thickness fringe pattern modified by wrinkles. Right panel: amplitude of membrane deflection in gray scale. The wrinkles make an angle of about 8° between them and the typical radius r_0 of the region in which they merge is of about 3 μm. Lower panel: profile of the wrinkles. Maximum vertical displacement along the wrinkles as a function of distance from the cell body. W_i indexes the wrinkle i on the top-right panel. The maximum amplitude A is of about 0.3 μm and the length L about 150 μm.

culture medium and fibroblasts. By imposing a small tilt of the second mirror, one produces regularly spaced parallel fringes which are distorted when the membrane wrinkles [Fig. 3(a)]. The contrast is increased by locating a drop of optical fluid on the second mirror of the interferometer and the wrinkles are better visualized when they are perpendicular to the fringes. The profile of each of the wrinkles, in a plane perpendicular to the sample plane, can be reconstructed from the local displacement of the fringes [Figs. 3(b) and 3(c)]. The vertical resolution achieved is about 25 nm whereas the spatial resolution is better than 5 μm.

In order to demonstrate the applicability of the method, let us now interpret, in the framework of our previous theoretical analysis of the problem,[9] the wrinkle pattern described in Fig. 3, which results from the contraction of a single fi-

broblast. We expect from the theory the wrinkle maximum amplitude $A \simeq \sqrt{h r_0}\sqrt{3[(\tau - T_0)/B]}$, where τ stands for the tension imposed by the cell in the adhesion region of typical size r_0. From the experimental A and r_0 (Fig. 3), we obtain $\tau \simeq 40$ mN/m or forces, $\tau r_0 \simeq 200$ nN, which are equivalent to those reported on Ref. 8. However, it should be noticed that the dynamical range for force measurement is limited. First, radial forces smaller than T_0 cannot be detected and second for a force twice T_0 the cell requires to contract by $r_0/3$ since B/T_0 is of the order of unity. As described in Ref. 8 this difficulty is overcome by tuning the membrane elastic properties by either increasing B (thicker membranes) or by weakening the membrane by the use of UV light. In conclusion, the protocol, which we proposed for the preparation and characterization of the membrane, provides us with the thickness $h \simeq 20$ nm, the tension $T_0 \simeq 27$ mN/m, and the elastic modulus $B \simeq 20$ mN/m, which together with interferometry methods allows us to measure, in situ, the field amplitude of a wrinkled pattern produced by living cells on a silicone substrate. Our optical method, in addition, might prove useful for a variety of related applications, for instance, the study of the evolution of the cell body itself.

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