The Wrinkling of Thin Elastic Membranes as a Cancer Diagnostic

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Abstract:

Cancerous cells generate wrinkles on sufficiently thin elastic membranes. Exploiting this phenomenon could enable the fabrication of a point-of-care bladder cancer diagnostic device. Historically, there has been difficulty producing thin, large membranes; while this remains a difficulty, a limited number of membranes were fabricated. Membranes as thin as 150 nanometers were produced. The wrinkles generated by bladder cancer cells, large enough to be observed under optical microscope, were characterized for each of these membranes.

Introduction:

Cell locomotion is dependent on the forces a cell exerts on its exterior environment, called cell traction forces. When this exterior environment is composed of an ultra-thin elastic membrane, these traction forces result in significant wrinkling of the surrounding membrane. As a cell attempts to move, it inadvertently draws the ultra-thin membrane towards localized focal points at the outer edge of the cell membrane, and the angular component of the stress in the surrounding membrane becomes negative. This angular compression results in a localized buckling of the membrane.

Because cancer cells produce distinctly different forces from healthy cells [1], this wrinkling phenomenon could be applied as a point-of-care cancer diagnostic device. One of the great strengths of such a device is that a heterogeneous sample of cells might be used for reliable diagnosis of cancer; for example, the cells found in urine samples could be used to diagnose bladder cancer.

Attempts to find a quantifiably testable predictor for the formation of wrinkles yielded a numerical study [2]. Fitting curves to the simulated data yielded equations predicting length and number of wrinkles based on the physical qualities of the membrane (radius, thickness, Poisson's Ratio, residual stress) and those of the cell (radius, force exerted by a half-cell). These equations are found in Figure 1, with R* as the wrinkle length, N the number of wrinkles, δ_c the critical membrane displacement for wrinkle formation (linearly related to idealized cell traction force), R the membrane radius, r_0 the cell radius, β the distance pulled at the boundary of the membrane (linearly related to residual stress), h membrane thickness, and σ Poisson's Ratio.

$$R^* = \sqrt{\frac{1-\sigma}{1+\sigma}\frac{\delta R+\beta r_0}{\delta r_0+\beta R}Rr_0} \quad \boxed{1}$$

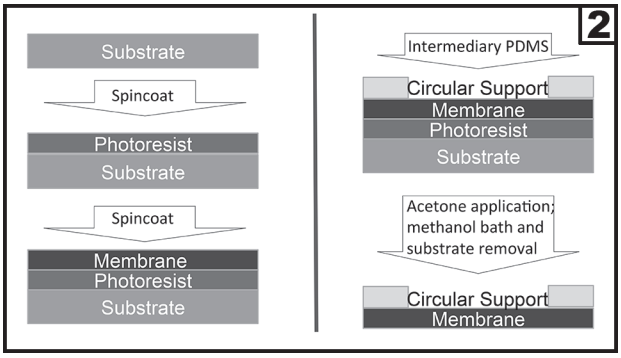
$$N=3/2500\,\beta r\downarrow 0\,/h\uparrow 2\ +69/400\,r\downarrow 0\,/h+1/50\,\beta/h+2$$

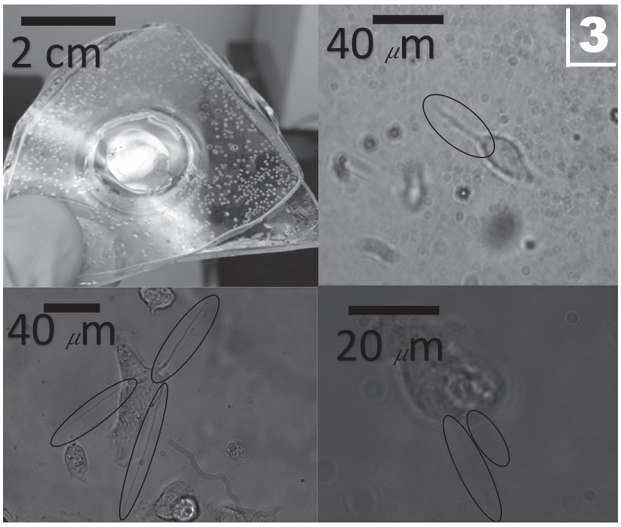
$$\delta_c=\frac{23}{20}\beta-\frac{1}{250}\frac{r_0\beta}{h}+\frac{131}{5}\frac{h^{5/3}}{r^{2/3}}+\frac{56}{10}h-\frac{19}{20}\ln(\frac{r_0}{h})$$

Experimental Procedure:

The membrane fabrication process is summarized in Figure 2. First, several microns of AZ 4330 photoresist were spun onto a 4-inch silicon wafer. After baking for one minute at 100°C, we placed hexane-diluted polydimethylsiloxane (PDMS) on top of the photoresist and again spun the sample, this time at 6000 rpm for three minutes. At this point, our sample was placed on a hot plate at 80°C for 16-18 hours.

After the PDMS membrane cured, a thick PDMS support structure was adhered to the sample via intermediary





PDMS layer and cured at 100°C for two hours. After cooling, the sample was treated with an acetone mist via spray bottle and subsequently immersed in a methanol bath. Careful peeling of the support structure resulted in the production of a bounded membrane, which could be seen to oscillate in the methanol. The membrane was then carefully removed from the methanol bath [3].

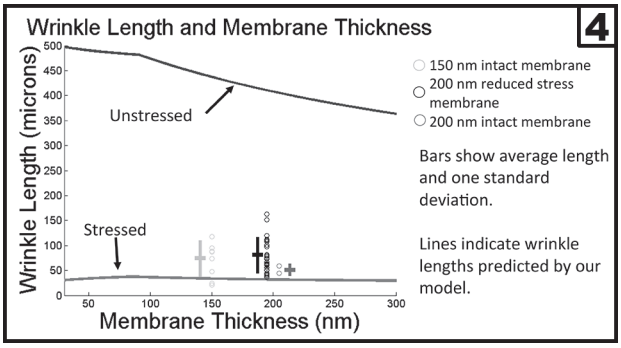
T24 bladder cancer cells were cultured. The cells were incubated on the membrane at 37°C in 5% CO_2 for 24 hours. Over this time period, cells adhered to the surface and contracted, creating the wrinkles observed via inverted microscope (Figure 3).

Results and Conclusions:

Five membranes were fabricated: 1 × 150 nm, 3 × 200 nm, and 1 × 300 nm. Membranes of each thickness were tested and T24 cells were unable to generate wrinkles in the 300 nm membrane. The length and number of wrinkles formed on the 150 nm, 200 nm, and 200 nm reduced-stress membrane after 24 hours were 70±35 microns and eight; 52±10 microns and two; and 82±33 microns and twenty-nine, respectively. After 48 hours, these quantities were: 40±27 microns and thirteen; 40±7 microns and two; and 85±54 microns and 26. The 24 hour data is summarized in Figure 4.

The reduced-stress membrane was a 200 nm membrane with hole in the center (roughly a quarter of the area of the membrane). This hole allowed the membrane to relax and relieve some of its residual stress, which likely accounts for the substantial increase in both length and number of observed wrinkles.

We were unable to reduce the membrane thickness below 150 nm. Although membranes as thin as 125 nm were observed in the methanol environment, removal of the membrane from the bath caused breakage.



While we did find an upward trend in length and number of wrinkles as membrane thickness was reduced, our results indicated that our membrane was stressed as a side effect of our fabrication process. This residual stress substantially increased the minimum force required for cells to generate wrinkles, and likely reduces the device's power to differentiate between cells producing different ranges of force.

Future Work:

In the future, we hope to refine our fabrication process to increase control over the residual membrane stress and thickness while improving membrane stability. This will allow for the statistical analysis of wrinkle formation by a variety of cells and an optimization of membrane characteristics for the fabrication of a point-of-care bladder cancer diagnostic device.

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