ADVANCED MATERIALS

Supporting Information

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Large-Scale, Ultrapliable, and Free-Standing Nanomembranes

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Large-scale, Ultra-pliable and Free-standing Nanomembranes and Measurement of Epithelial Monolayer's Modulus

Supplementary Text

Table S1 to S4

Supplementary figure 1 to 6

Supplementary Text

Preparation of the AFM Sample (AFM images for determining the membrane thickness)

The PDMS nanomembrane thickness was measured by placing a membrane on a silicon wafer (**Figure S2**). The sample membrane was immersed in liquid nitrogen, and then scratched using a sharp needle. The height profile was measured across the scratched region. AFM measurements were carried out using an XE-100 instrument (Park System, Korea).

Preparation of the SEM samples

PDMS nanomembranes were transferred onto porous alumina membranes. A membrane mounted on an anodisc was covered with another anodisc to form a sandwich-like structure. The sample sandwiched by the anodisc was immersed in liquid nitrogen to permit quick freezing, then chopped up using a cutter to obtain a cross-sectional image. The cross-sectional SEM images allowed the direct measurement of the membrane thickness, and a JEOL-6701F SEM (Japan) instrument was used to obtain images.

Ant walking experiments on the membrane

Onto a free-standing membrane (100 nm thick, diameter: 2.3 cm) was placed an ant (Lasius Niger, weight: 1.4 mg, length: ≈ 2.5 mm). A CCD camera was used to observe the ant's movements.

Preparation of samples for ATR FT-IR: Chemical bonding structure

The chemical differences between the bulk PDMS and the FSUT PDMS membranes were measured using a Spectrum100 FT-IR spectrometer (Perkin Elmer, UK). Four sample types were characterized: pure PDMS, 5 mm thick, and PDMS membranes 500 nm, 200 nm, or 100 nm thick. Each of the four samples was placed on the sample mount of the ATR-FTIR

instrument prior to the measurements.

Contact angle measurements

A 6μL water droplet was placed on the membrane surface, and the contact angle was measured using an EasyDropinstrument (DSA 15, KRÜSS, Germany). This experiment was repeated 5 times per membrane sample.

Water & IPA droplet test

Water was dropped onto the center of a membrane. Forty microliter droplets were deposited drop-by-drop using a micropipette until the membrane tore (tear point: 800μL, **Figure S4(a)**). The membrane droplet shape was monitored using a camera (NEX-5, Sony, Japan). **Figure S4(b)** (i-iv) shows that the membrane gradually extended (isotropically and anisotropically) as the water drop volume increased.

Mechanical properties of hydrophilic membrane

Two types of membranes of 70 nm and 500 nm thick were exposed to O_2 plasma with RF power of 20W for 5, 30 and 60 sec respectively, and then mineral oil were dropped onto free standing plasma treated membrane. We monitored the membrane extension by increasing oil volume and calculated the residual stress and young's modulus using the curvature of the extended membrane. **Figure S4(c)** shows the images of the 70 nm membranes extension under 1 ml mineral oil after plasma exposure for each 5 sec, 30 sec or 60 sec. **Table S4** shows the residual stress and young's modulus obtained by various conditions.

Calculation of Young's modulus for the PDMS membrane

The tensile stress and modulus were measured by forming pendant droplet-shaped membranes, modeled as a sphere, as illustrated in **Figure S5(a)**. Here, we assumed that the

membrane was uniformly stretched in the radial direction under the force of a water droplet. The tensile stress in the radial direction was obtained by expressing the body force of the water droplet across the thickness and circumference of the membrane.

$$\sigma = \frac{F_{B.F}}{\pi D \cdot t} = \frac{\Delta \rho V g}{\pi D \cdot t} \qquad (1)$$

Here, σ is the tensile stress in the radial direction of the membrane, $F_{B.F}$ is the body force of the water drop, D is the diameter of the ring, t is the thickness of the membrane, $\Delta\rho$ is the density difference between two fluids, V is the volume of water, and g is the acceleration due to gravity. The geometrical features can be used to express the tensile strain by dividing the extended boundary of a thin membrane by the initial diameter of the ring,

$$\varepsilon = \frac{\Delta L}{D} = \frac{L - D}{D}, \qquad (2)$$

Where ε is the strain in the radial direction of the membrane and L is the length of the extended membrane boundary (front view).

Thus, the tensile modulus of a thin membrane was obtained by substituting Eq. (1) and (2) into Hooke's Law as follows:

$\sigma = G\varepsilon$ Hooke's Law,

$$G = \frac{\sigma}{\varepsilon} = \frac{\Delta \rho V g}{\pi D \cdot t} \times \frac{L_0}{L - L_0}. \qquad (3)$$

Finally, the tensile modulus of a thin membrane, G, can be defined using Eq. (3).

MDCK cells cultured on a nanomembrane

The MDCK epithelial cell line (Korean Cell Line Bank) was used in this experiment. Cells were maintained in a T75 flask and were cultured in medium consisting of 10% Fetal Bovine Serum (FBS, Invitrogen, CA) in Dulbecco's Modified Eagle Medium (DMEM, Invitrogen, CA). Cells were passaged every 3 days by dissociating the cells into single cells using Tryp LE (Invitrogen, CA) and replating in a T75 flask at a subculture ratio of 1:5. The MDCK cell

suspension (adjusted to 2×10^4 cells/mL) was directly seeded onto a fibronectin-coated nano-membrane, and cells were incubated at 37°C for 3 days in a 5% CO_2 incubator.

Immunostaining analysis

MDCK cells grown on the nanomembrane were analyzed immunocytochemically to confirm the spatial distribution of the tight junctions. The cells cultured for 3 days were fixed for 20 min with 4% formaldehyde at 4°. Cells were permeabilized using 0.1% triton-X100 in 0.1% PBS for 20 min at room temperature, blocked with 3% BSA in PBS for 30 min, then incubated with primary antibody overnight at 4°. Primary antibodies (Invitrogen, CA) against the following proteins were used to characterize the various cell types: ZO-3 (1:250) was localized to the sites of cell–cell interactions, which coincided with the tight junctions. After incubating overnight, each nanomembrane was washed with PBSA (0.1% BSA in PBS) for 5 min. Secondary antibodies (1:1000 dilutions, Invitrogen, CA) were applied for 1.5 h at room temperature. Each nano-membrane was washed with PBSA, and fluorescent images were acquired using a confocal microscope (Olympus) after counterstaining with 4,6-diamidino-2-phenylindole dihydrochloride (DAPI, Invitrogen, CA).

Calculation of the Young's modulus for a cell membrane

Before evaluating the mechanical properties of the cell monolayer, we measured the thickness of the cell monolayer on the nanomembrane. Z-plane images of the MDCK cell monolayer were collected from 0 μm to 15μm in steps of 0.5μm using confocal microscopy, and stacked images were reconstructed. The thicknesses of the cell monolayer were measured by staining the cell–cell junctions and the centers of the cells using different antibodies (Actin-Green, ZO-3-red). At the center of the cell monolayer, actin and ZO-3 were distributed over 0–12μm and 0–10μm, respectively. The actin and ZO-3 were distributed over 0–9 μm at the cell–cell junctions. This result indicated that the thickness of the cell–cell junctions (10

μm) may be smaller than the thickness at the center of the cell (12μm), in agreement with previous studies [1]. Here, we assumed that the thickness of the cell monolayer was 10μm, which was the average value calculated from the thickness of the cell–cell junctions and the centers of the cell (n=15). The mechanical properties of the cell monolayer were evaluated by eliminating the tensile modulus of the membrane from the combined tensile modulus of the cell monolayer and the membrane shaped by the water droplet.

$$G_c = \frac{G_m t_m (\varepsilon_2 - \varepsilon_1)}{t_c \varepsilon_1} \quad (7)$$

where, G_c is the tensile modulus of the cell monolayer, G_m is the tensile modulus of the membrane, ε_1 is the strain in the radial direction of the membrane cultured with cells, ε_2 is the strain in the radial direction of the membrane, t_c is the thickness of the cell monolayer, and t_m is the thickness of the nanomembrane.

Table

Table S1 Young's modulus of various basement membranes (BMs) and soft tissue reported in previous studies. Most of the data in the table were obtained by AFM or hydrostatic pressure methods applied to isolated tissue [2-8]

	Vascular endothelial BM	***Human corneal BM***	***Retinal BM***	***Renal tubules***	***Capillaries***	***Alveolar capillary***	***Alveolar sheet***
Young's modulus	*8–70 kPa*	*2–80 kPa*	*0.95–3.30 MPa*	*Low 2-5 MPa High 20 MPa*	*Low 2-5 MPa High 20- 30 MPa*	*2-20 MPa*	*3 MPa*
Thickness	-	-	*402 nm*	*100-250 nm*	*40–100nm*	*100 nm*	*100 nm*
Measurement method	*AFM*	*AFM*	*AFM*	*Pressure diameter-length measurement*	*Pressure diameter-length measurement*	*Pressure diameter-length measurement*	*Pressure diameter-length measurement*
References	***3***	***4, 5***	***6***	*7*	*7*	***7, 8***	*7*

Table S2 The diameter and thickness affected the rate of successful detachment of the PDMS nanomembrane from the substrate. Obtaining free-standing PDMS nanomembranes(less than 100nm thick) remains a challenge.

Mixing ratio (PDMS:Hexane)	***Thickness (nm)***	***Diameter(cm)***			***Successful separation rate (%)***
		2	***3.5***	***6***	
1:5	*2000*	o	o	o	*100*
1:10	*1300*	o	o	o	*100*
1:20	*650*	o	o	o	*90*
1:30	*500*	o	o	o	*90*
1:40	*373*	o	o	o	*90*
1:50	*207.5*	o	o	o	*80*
1:60	*150*	o	o	o	*80*
1:70	*148.8*	o	o	-	*70*
1:80	*130*	o	o	-	*70*
1:90	*113.25*	o	o	-	*50*
1:100	*92.3*	o	o	-	*50*
1:120	*77*	o	-	-	*30*

Table S3 Comparison of the Young's moduli reported in three reference papers. The chart shows the Young's modulus measurement methods, the membrane thickness, materials, and characterization presented in each of the three papers. Each measurement was associated with some limitations. The highest Young's modulus (7.76 MPa) was measured by *M. R. Glucksberg*, et al. [9], but, their membrane was non-uniform and the Young's modulus exceeded the manufacturer's specification.

	Glucksberg, 2007	***Chen, 2009***	***Mofrad, 2010***
Dimension	*287 ~ 986 μm (3 μm)* *315 ~ 723 μm (492 nm)* *(Circular membrane)*	*Dog-bone shaped test sample* *(length 6.6 mm)*	*286 μm ~ 1.8 mm*
Young's modulus	***7.76MPa (492nm),*** ***6.61MPa (3μm)***	***0.6MPa ~ 1.4MPa***	***0.45 MPa (11.8μm)***
Thickness	*492nm, 3μm*	*50μm ~ 1800μm*	*11.8μm, 22.8μm*
Material	*Hexane + PDMS*	*Pure PDMS*	*Pure PDMS*
Measurement Method	*Bulge test*	*ASTMD 412 test*	*Bulge test*
Characterization	*Young's modulus*	*Shear stress effects*	*Two Mooney–Rivlin constants*
Limitation	*- Non- uniformity of the membrane* *- Unconvincing data*	*Micro-size membrane (PDMS polymer chain ~ 10nm)*	*Micro-size membrane*

Table S4 Residual stress and young′s modulus of plasma exposed membranes.

Thickness	***Time for the Plasma exposure*** **[*sec*]**	***Contact angle*** **[°]**	***Residual stress*** **[*MPa*]**	***Young's modulus*** **[*MPa*]**
70 nm	*Non treatment*	*110 ± 10*	*0.14 ± 0.08*	*0.44 ± 0.078*
	5 sec	*70.7 ± 5*	*0.25 ± 0.026*	*7.28 ± 0.75*
	30 sec	*26.1 ± 3*	*1.06 ± 0.11*	*107.80 ± 24.00*
	60 sec	*19.8 ± 3*	*1.36 ± 0.28*	*184.13 ± 44.78*
500 nm	*Non treatment*	*110 ± 10*	*0.05 ± 0.005*	*2.01 ± 0.15*
	5 sec	*70.7 ± 5*	*0.06 ± 0.008*	*5.14 ± 0.15*
	30 sec	*26.1 ± 3*	*0.28 ± 0.025*	*128.06 ± 29.67*
	60 sec	*19.8 ± 3*	*0.34 ± 0.036*	*149.90 ± 56.10*

Supplementary figures

Figure S1 Schematics of the fabrication processes of the PDMS nanomembrane: (a) Schematic diagram of the two layers (AZ1512 as a sacrificial layer, hexane-PDMS as a solution) present during spin-coating; explanation for how the spin-coating process introduced shear forces that formed the UT PDMS membranes. (b) A PDMS block was used to handle the membranes once they were free-standing (left). Acetone spread across the membrane quickly, passed through the membrane, and dissolved the AZ1512 sacrificial layer. Immersion in methanol easily and smoothly detached the membrane. The surface tension affected the membrane shape once a water droplet had been placed on the membrane (right).

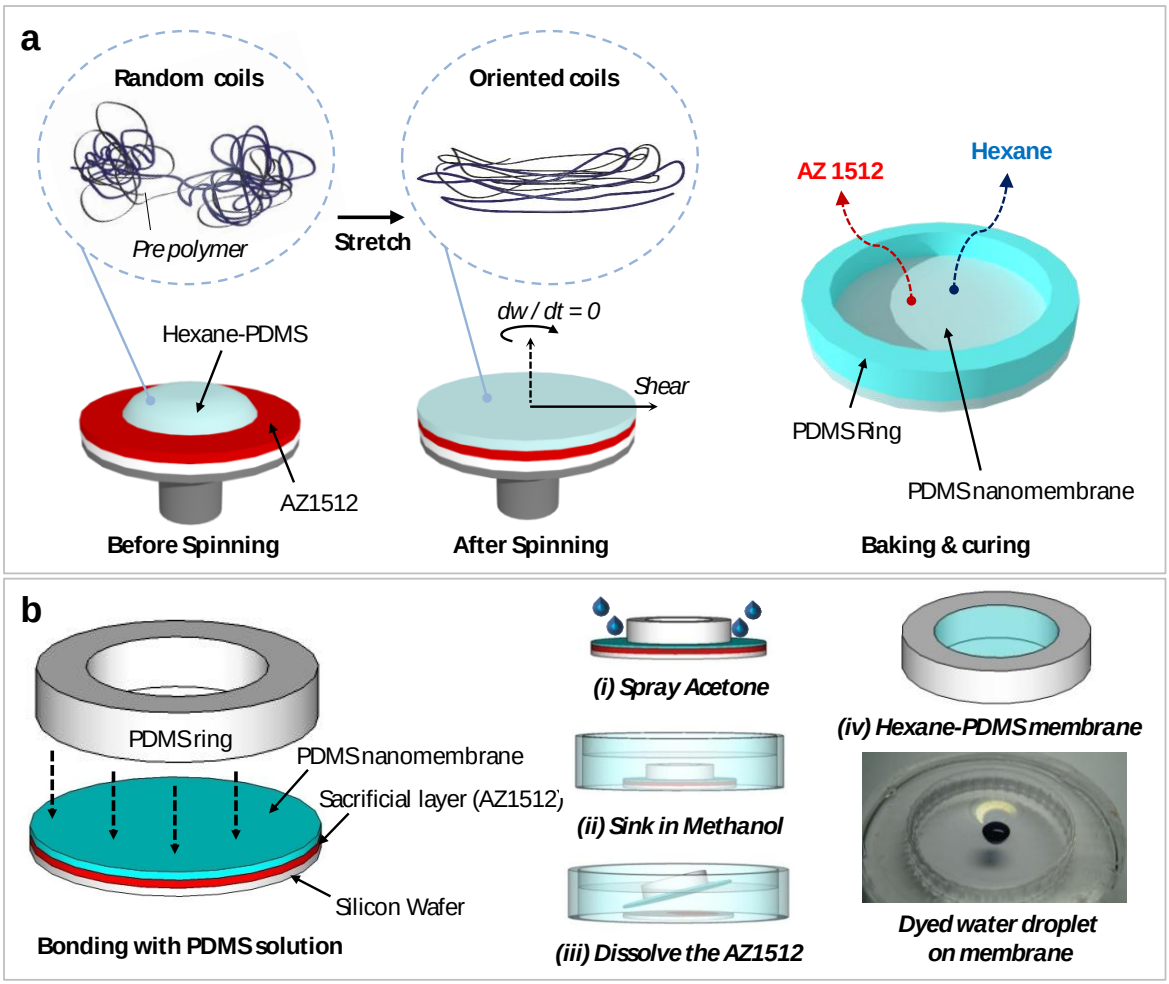


Figure S2 AFM measurements of the membrane thickness as a function of the PDMS: hexane ratio (left). Height analysis of the profile indicated in the AFM images of different membranes (right).

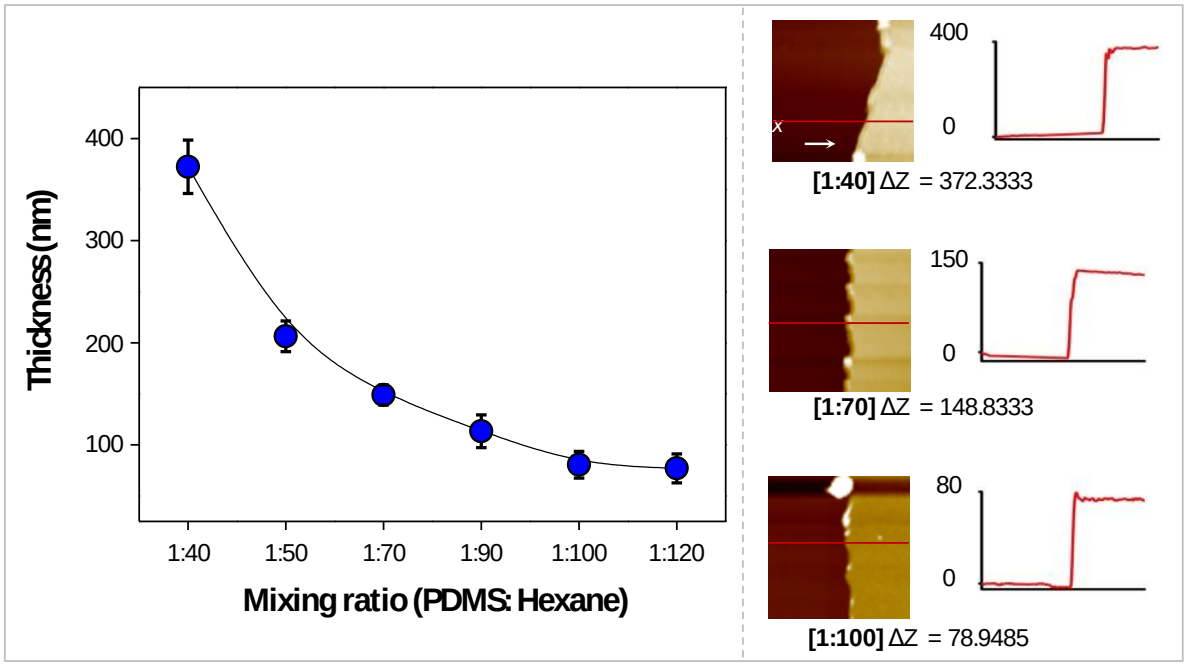


Figure S3 Recovery of the hydrophobic properties after oxygen plasma treatment of the membrane: (a) Images of a 70 nm membrane, PDMS (bulk) with a 6 µL water droplet as a function of time elapsed after plasma treatment (b) The plot indicates the hydrophobic surface recovery for 3 types of sample as a function of the time elapsed after oxygen plasma treatment. Scale bar indicates 2 mm in **a**.

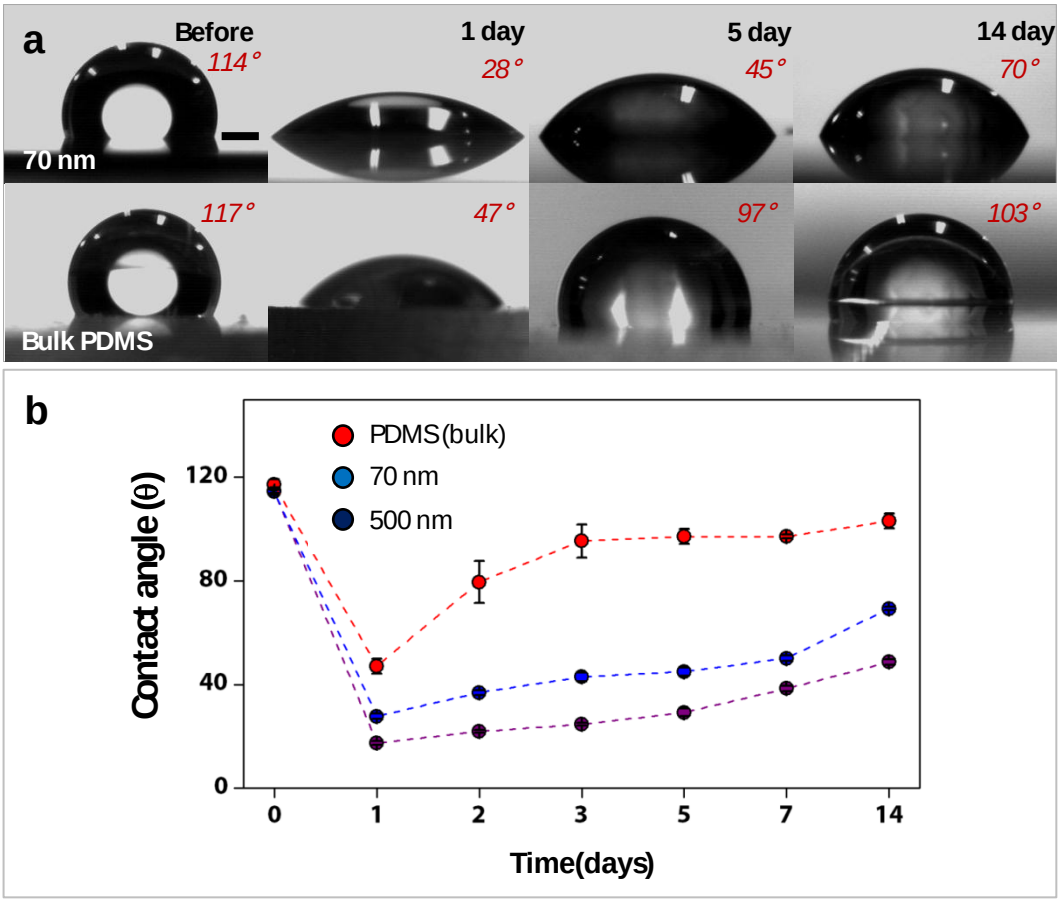


Figure S4 Polymer changes in the membrane upon addition of a water droplet: (a) Images of blue-dyed water droplets with volumes of 80 μL, 240 μL, 400 μL and 720 μL; (b) Schematic diagram of the polymer chain as a function of the water droplet quantity. Polymer chains became elongated, and their alignment occurred during membrane stretching. (c) Images of a 1 ml droplet of mineral oil on the plasma exposed membranes, 5 sec, 30 sec and 60 sec respectively. Scale bar indicates 5 mm in **a**.

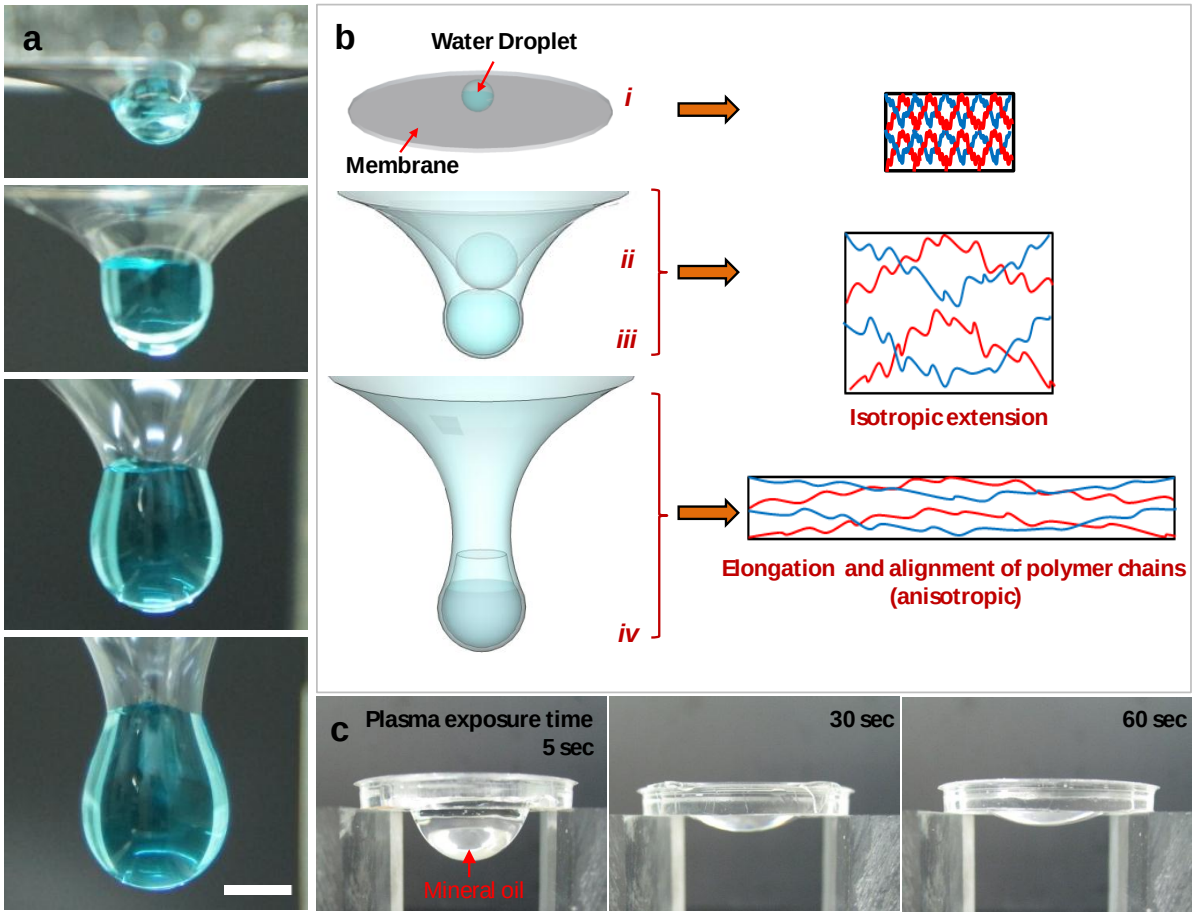


Figure S5 Mechanical properties of the membrane: (a) Geometries used in the calculation of the young's modulus for the PDMS nanomembrane; (b) Strain–tensile stress curve for membranes of three thicknesses: 500 nm, 200 nm, and 70 nm.

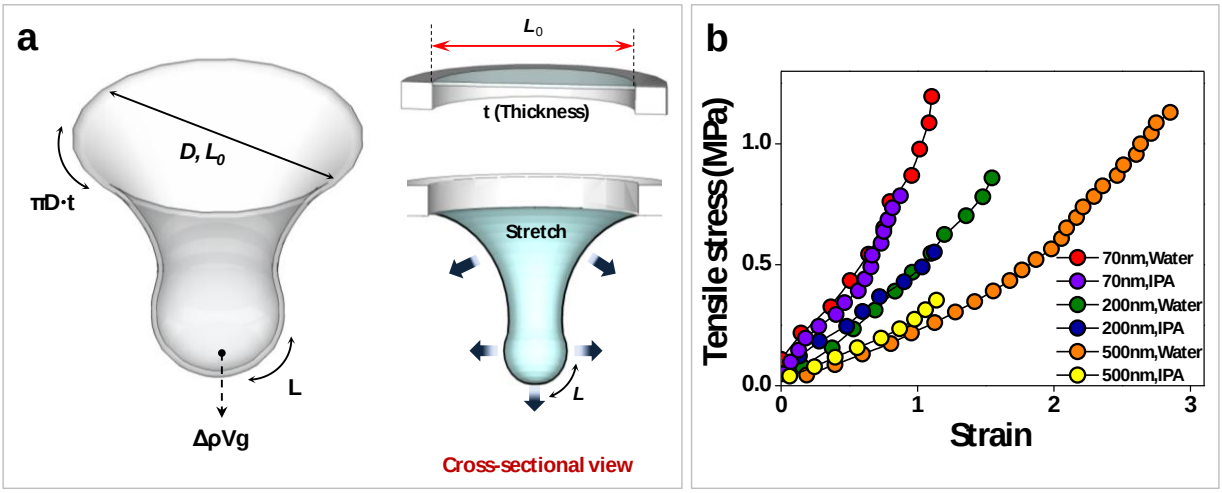
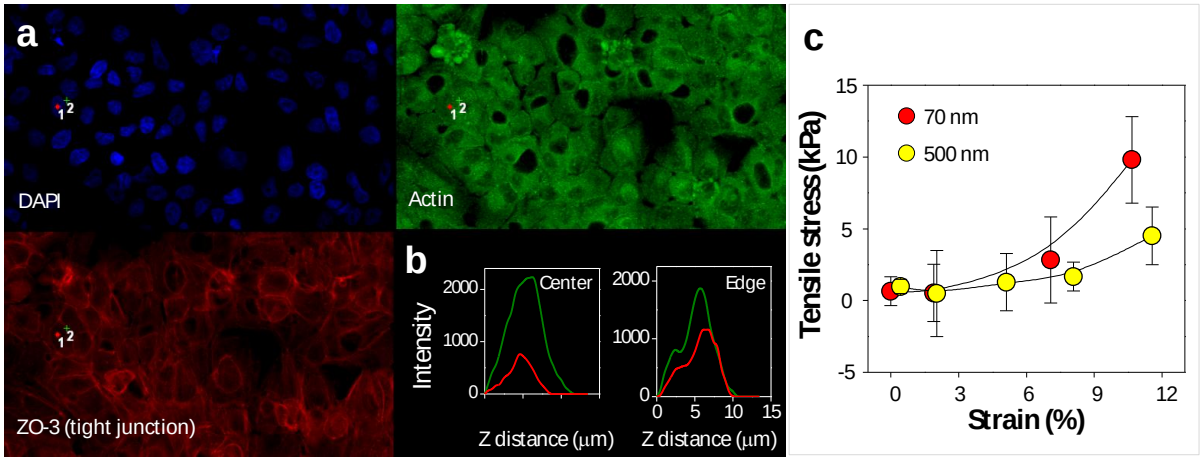


Figure S6 Thickness measurement of a cell monolayer on a nanomembrane: (a) Stacked images at different positions on the monolayer, imaged by staining with DAPI, Actin, and ZO-3; (b) Plots of the fluorescence intensity as a function of displacement across the different levels (Z): at the cell–cell junctions and at the center of a cell (Actin, ZO-3). (c) Tensile stress and strain for a cell monolayer on the order of 0 - 10 %.



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Video 1 (V1): shows the real time detachment of a 100 nm membrane from the Si wafer in a methanol bath. The sacrificial layer was rapidly dissolved by acetone and methanol, which enabled the smooth detachment of the nanomembrane from the wafer.

Video 2 (V2): shows the elasticity of a 100 nm membrane. The membrane stretched upward to 3.5 cm by pulling a paper tissue adhered onto the membrane surface (diameter of membrane: 3.5 cm)

Video 3 (V3): shows an ant walking on a 100 nm membrane. The membrane was partially extended by an ant's leg (weight: 1.4mg).

Video 4 (V4): shows the deformation of the 100 nm membrane as the volume of water increased. 40 μL of blue-dyed water was dropped onto the membrane each time; time goes 4 times as fast in the video.