Electronic Supplementary Material (ESI) for Lab on a Chip.

SUPPLEMENTARY INFORMATION

SI materials and Methods

Calibration sample protocol
Silicone membranes (MED4086) of known elasticity were used as calibration samples. The calibration samples were attached to half of the measurement membrane by simply adding a small ethanol drop before bringing the two surfaces into contact. After two hours drying the two membranes, they showed strong adhesion between them. The Young's modulus measured within the proposed device was then compared to the one measured with a commercial pull-tester device.

Immunofluorescence protocols
Cells cultured on membranes were treated with 0.4% Triton-X, 0.4% formaldehyde solution for 3 minutes. They were then fixed in 4% formaldehyde for 20 minutes, permeabilized in 0.4% Triton-X for 10 minutes and finally blocked in 5% BSA (bovine serum albumin) for 2 hours. The primary antibody was added (α-Vinculin or α-ZO-1 produced in mouse at 1:100 concentration) and incubated overnight at 4°C. A secondary antibody is then incubated (α-mouse, 1:500) for 2 hours and finally Phalloidin-488 (1:20) was added for 45 minutes. The membranes were mounted on glass slides using DAPI mounting medium for long term storage. Images were taken at a confocal microscope Leica (TCS SP5) and a fluorescence microscope (JPK Microscope Axiovert 200, Zeiss).

Cell culture protocols
Madin-Darby Canine Kidney (NBL-2) (MDCK) were cultured in Eagle's Minimum Essential Medium (EMEM) supplemented with 10% inactivated fetal bovine serum. Sarcoma osteogenic (SaOS2) were cultured in DMEM medium supplemented with 10% inactivated fetal bovine serum and 1% L-glutamine.
Live cell fluorescence was performed by incubating 2 drops of NuncBlue (Hoechst 33342 dye, Thermo Fisher Scientific) per medium ml for 20 minutes and then directly observing the cell under a fluorescence inverted microscope (JPK Microscope Axiovert 200, Zeiss).

Cell thickness measurement
Confocal imaging (Leica confocal microscope) of the cell layer on the membrane from a z-stack used to measure the thickness of the cell layer. In the picture MDCK layer is shown with green staining for Alexa 488, red for tight junction protein ZO1 and blue for DAPI. Performing a linear cut of the z-stack allows to estimate the thickness of the layer in the order of (10 ± 2) µm.

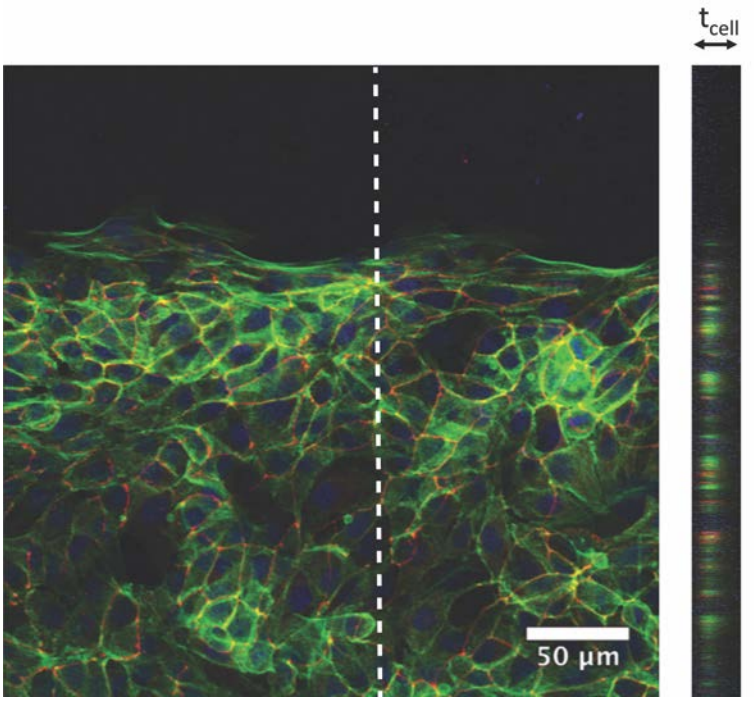


Fig.S1: Confocal imaging (Leica confocal microscope) of the cell layer on the membrane from a z-stack used to measure the thickness of the cell layer. In the picture MDCK layer is shown with green staining for Alexa 488, red for tight junction protein ZO1 and blue for DAPI. Performing a linear cut of the z-stack allows to estimate the thickness of the layer in the order of (10 ± 2) µm.

Force measurement and Young's modulus calculations

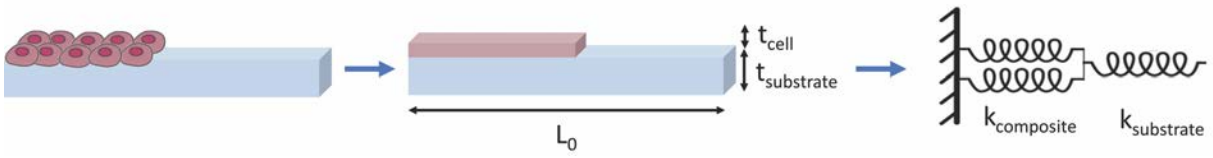


Fig.S2: Schematic of the cell layer and substrate model in the differential approach: the same force is exerted on both regions as in two springs in series, they therefore deform of different amount depending on their Young's modulus.

The bare region and the cell covered region are subjected to the same force (Fig.S2):

$$F_{bare} = F_{composite} \Rightarrow \sigma_{bare} t_{bare} = \sigma_{composite} t_{composite} \quad (1)$$

Using the stress and strain relation it is possible to rewrite the above formula as:

$$\varepsilon_{bare} E_{bare} t_{bare} = \varepsilon_{composite} E_{composite} t_{composite} \quad (2)$$

In the cell region, as the cells deform with the substrate, they are in iso-strain conditions. No mechanical component was needed to model the cell to substrate adhesion as from our observations the cells were tightly anchored to the substrate (Fig.3c). In this configuration, the Young's modulus of the composite material can be measured using the rule of mixture:

$$E_{tot} = E_1 f + E_2 (f - 1) \quad (3)$$

Where f represents the volume fraction of layer 1 and 1-f the volume fraction of layer 2. Assuming that cell uniformly cover the membrane, the only difference in geometry is given by the difference in thickness between the layers, and eq.3 can be simplified as:

$$E_{tot}(t_{substrate} + t_{cells}) = E_{substrate} t_{substrate} + E_{cells} t_{cells} \quad (4)$$

Combining equations (2) and (4) it is possible to obtain:

$$E_{cell} = \left(\frac{\epsilon_{bare}}{\epsilon_{composite}} - 1 \right) E_{bare} \frac{t_{bare}}{t_{cell}} \quad (5)$$

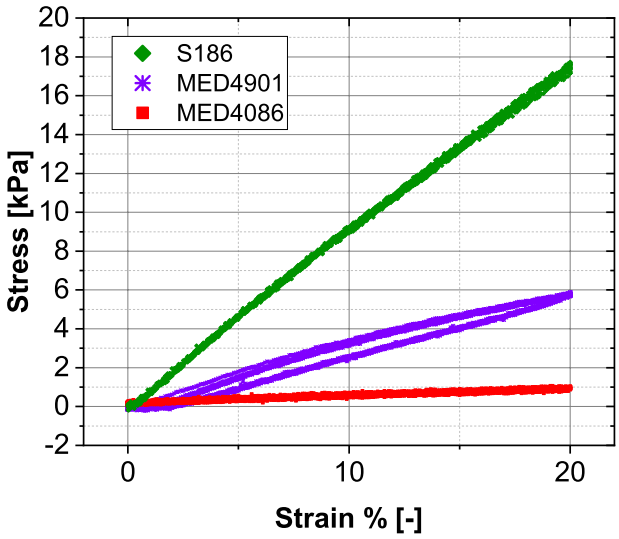
The Young's modulus of the cell can therefore be measured from the ration between the strains, the Young's modulus of the bare substrate and the ration in thickness between the two layers (considering that the widths are the same).

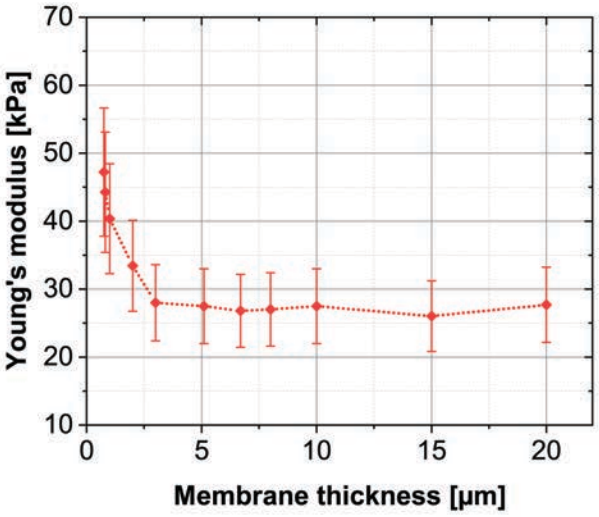
Material characterization and membrane design

The optimal PDMS formulation for ensuring adequate membrane sensitivity was chosen among several commercially available formulations from their mechanical characterization.

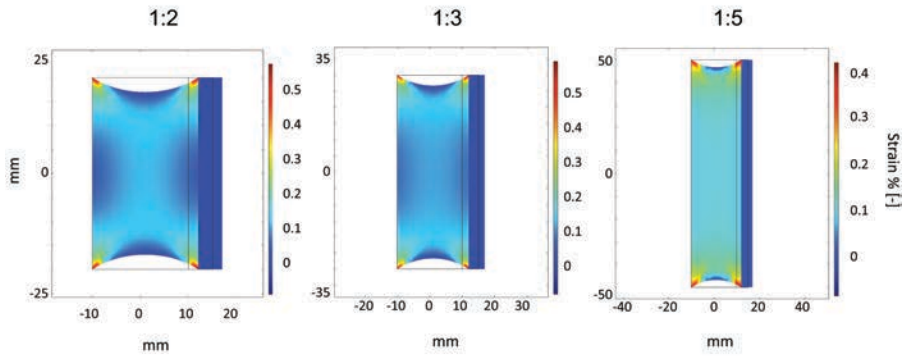
The Young's modulus of suspended membranes at different thickness has been measured for choosing the optimal membrane thickness. Thickness from 750 nm to 20 µm have been analyzed. The results show that the mechanical properties are constant for membrane with a thickness above 3.5 µm, and then rapidly increase when the thickness are below 3.5 um. There is therefore no advantage in lowering the thickness of the PDMS membrane below the 3-4 µm range. The thickness is therefore fixed in a range between 5 and 10 µm which allows to combine low Young's modulus with low thickness and thus increase the measurement sensitivity.

The membrane geometry was chosen taking into account the following factors: limiting the lateral necking on the sides during deformation, strain uniformity and miniaturization of the membrane and the set-up. By increasing the membrane aspect ratio, it is possible to minimize the lateral necking and obtain a good strain uniformity. On the other hand, high aspect ratio membranes can be challenging to fabricate and integrate in miniaturized measurement device. The surface strains at different membrane aspect ratio were compared using COMSOL Multiphysics FEA (Finite Element Analysis) simulations. Rectangular membrane of 1:2, 1:3 and 1:5 aspect ratios undergoing a 10% deformation were considered. We concluded that a 1:5 aspect ratio can
combine strain uniformity and dimensions compatible with standard equipment (sample dishes size, optical imaging field of view...).





(a)



(b)

Fig.S3: The design of the membrane is based on mechanical properties characterization and COMSOL Multiphysics FEA simulations: (a) Characterization of different commercially available PDMS formulations (Instron Single Column Universal Testing System, 3340) and relationship between Young's modulus and membrane thickness for MED4086 based suspended membrane. An increase of stiffness below 3 µm thickness membrane is reported due to the transition from the bulk material properties to the surface properties (measurements averaged on 3 samples per point). (b) Design comparison using COMSOL Multiphysics FEA simulations. Different aspect ratios are compared in terms of their surface strain uniformity when a 10% deformation is applied. The 1:5 design was selected as optimal geometry because of the strain homogeneity combined with fabrication feasibility.

Membrane preparation

The membrane is made of the PDMS formulation MED4086 and is fabricated using a casting machine. After curing of the membrane on the PET foil, (3x15) mm rectangular shapes are cut and attached to custom laser-cut frames. The membranes are then pre-stretched of 1.5 stretch (50% strain) to keep them in tension and avoid sagging. The pre-stretch is also necessary for the cells to better adhere and spread on the surface. After the pre-stretch, a new PET frame is attached to the suspended membrane to keep it in tension (Fig.S4).

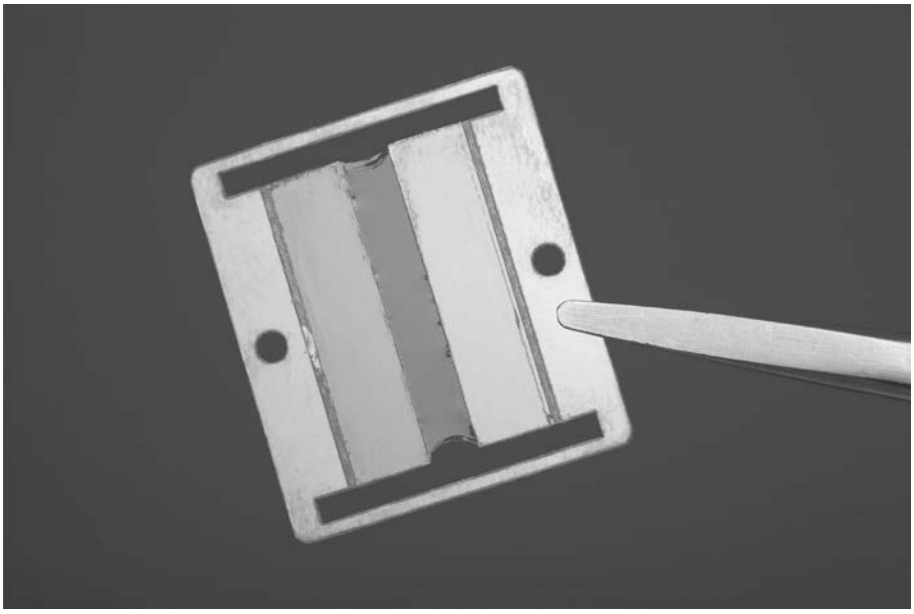


Fig.S4: Picture of a 1:6 (here 2x12 mm) aspect ratio suspended membrane on a PET frame holder after release and pre-stretch. Membrane fabrication process: PDMS uncured solution is spread at the desired thickness on a PET foil using a blade caster. This method allows to have relatively big area covered with PDMS (and high reproducibility of the membrane thickness). After curing in the oven, the membranes can be released from the PET foil and have them suspended on PET frames by water immersion.

EDTA chemical treatment

The choice of the EDTA treatment incubation time was made by comparing confocal images of the tight junctions at 0, 30 minutes and 1 hour incubation (Fig.S5). Longer incubation times have been discarded for the possibility to harm the cells. After 1 hour time incubation the junctions are almost completely disrupted in comparison to a control sample, however the cells still adhere to the substrate.

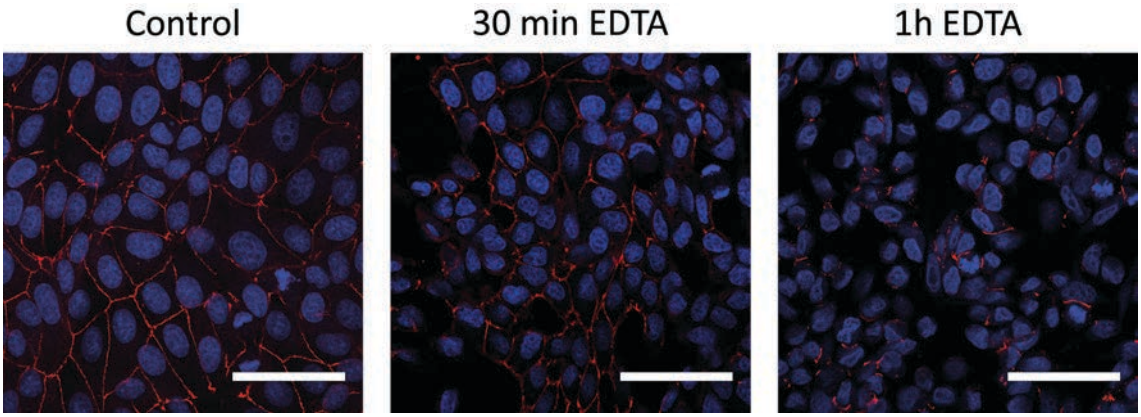


Fig.S5: Confocal images of MDCK cultured on a suspended PDMS membrane at different EDTA incubation times. In the control figure the tight junctions (red) are well developed and are uniformly present, after 30minutes EDTA incubation, cellular tight junctions start to be damaged and at 1 hour incubation time they are almost completely disrupted. (s.b. = 50 μm).

Control experiments performed in the same conditions but in absence of cells confirm that the chemicals used to stimulate changes within the cells do not affect the membrane mechanical properties (Fig.S6).

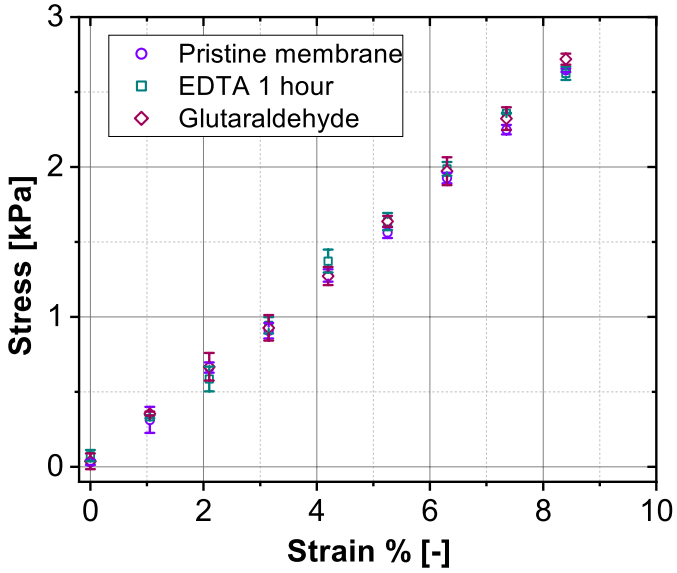


Fig.S6: Stress versus strain curves comparing the response of pristine PDMS membrane and after EDTA and glutaraldehyde treatments (3 samples each). These two chemicals do not affect the mechanical properties of the PDMS membrane.

Cell culture comparison – PDMS suspended membranes and polystyrene standard dishes

In order to investigate the effect of the substrate on cellular growth and morphology, cells cultured on the suspended membrane were compared to cells cultured on standard plastic dishes. We could not observe substantial differences between the two regarding the morphology of the cells.

From our observations we therefore concluded that cells can healthily grow on our membrane despite their low thickness and Young's modulus.

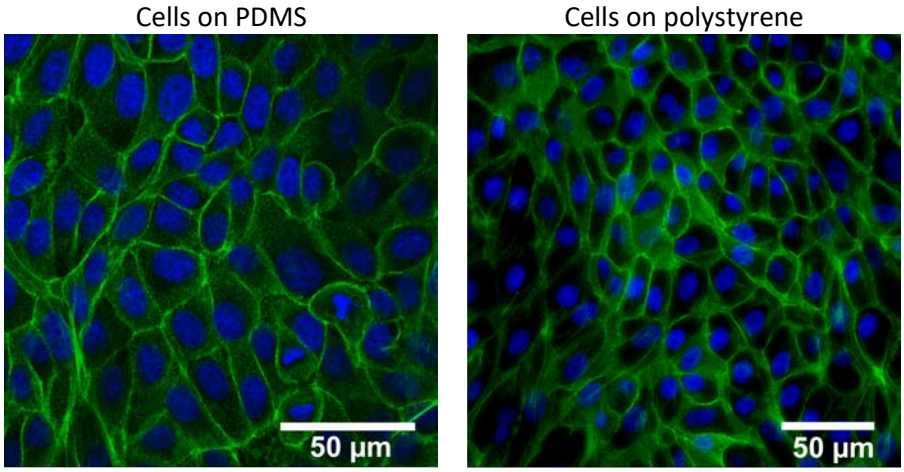


Fig.S7: MDCK cells cultured on our thin suspended PDMS membranes (left) and standard plastic dishes (right). The cells were stained using actin antibody (green) and dapi (blue).